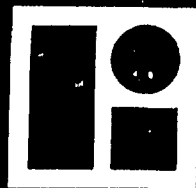


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6 Mammalian Toxicological
Evaluation
of DIMP and DCPD
(Phase 2)

9 FINAL REPORT
on phase 2

By
10 E. Ross/Hart, Ph.D.

11 March 1980

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Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Washington, D.C. 20314

15
Contract No. DAMD 17-77-C-7003

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Fort Detrick, Frederick, Maryland 21701

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In the rat over three successive generations with two matings per generation. Nerve fiber degeneration (demyelination) in chickens treated once at 1500 mg/kg or twice at 1000 or 500 mg/kg. No toxic effects were seen over a 90-day period in beagles given dietary mixes containing 150, 1500 or 3000 ppm of DIMP.

Purified DCPD proved non-mutagenic. No teratologic effects were observed in rats given dietary levels of 80, 250 or 750 ppm on days 6 through 15 of gestation. No deleterious effects on reproductive processes were seen with two matings per generation. No important evidence of toxicity was seen in beagles given 100, 300 or 1000 ppm in their diet over a 90 day period.

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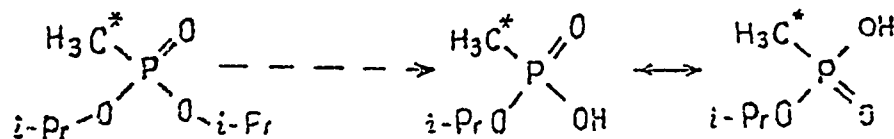
EXECUTIVE SUMMARY

DIMP

The test compound, DIMP USAMBRDL EPRD-1, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

The test compound, DIMP Lot AF-74, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Greater than 90% of the radioactivity of administered labelled diisopropyl methyl phosphonate (DIMP) to rats, mice and dogs was recovered in the urine. In all three species about 95% of recovered radioactivity comes from a single highly polar component. This component is identified as isopropyl-methyl-phosphonic acid (IMPA). The identification is based on comparing the metabolite with authentic IMPA by three different chromatographic techniques; namely, thin layer chromatography, ion chromatography and gas liquid chromatography coupled with mass spectrometry.



The test material was administered in the diet at doses of 100, 300 and 3000 ppm to pregnant female rats on days 6 through 15 of gestation. There were no changes in the dams or among the fetuses that indicated an adverse compound-related effect.

Dietary incorporation of DIMP at levels of 300 and 3000 ppm produced no dose-related reproductive response in the rat for three successive generations with two matings per generation. The first mating of the third generation resulted in significant pup losses from Day 4 to Day 21 for the treatment groups. This decrease was not evidenced for the second mating. Food consumption and body weight data for the parent (F0), second generation (F1b) and third generation (F2b) rats, indicated statistical equivalence with the controls for all treatment levels except the high dose F2b female group. Litter and necropsy observations for the first breeding of the three generations were unremarkable and free of any dose-dependent relationship.

Treatment of atropinized White Leghorn hens with DIMP once at 1500 mg/kg or twice at 1000 or 500 mg/kg did not produce evidence of nerve fiber degeneration after 21 days (animals treated once) or 46 days (animals treated twice) when the sciatic nerve was examined microscopically. Evidence of unsteady gait was easily distinguished clinically from signs exhibited by the positive control animals and was judged to be unrelated to nerve fiber degeneration.

The positive control animals, treated with TOCP once at 500 mg/kg, developed classical progressive clinical signs of nerve fiber degeneration beginning 12 to 14 days after treatment. Most of the animals showed microscopic evidence of nerve fiber degeneration.

Beagle dogs, four per sex per group, were given diisopropylmethylphosphate (DIMP) in the diet for 90 days. Dietary concentrations were 150, 1500 and 3000 ppm, and a control group was maintained in parallel. Initially and at 4, 8 and 13 weeks hemograms and clinical chemistry values were obtained on all dogs. The dogs were examined daily as to general condition, and weekly body weights and food consumption data were obtained. An ophthalmologic examination was conducted initially and at 13 weeks. At termination each dog was grossly necropsied and approximately 27 tissues were preserved. Eight organs were weighed. Tissues from the control and high-level groups were examined histologically. The dogs continued in good general health throughout the study. No clear or meaningful changes were seen in the data collected that could be ascribed to the ingestion of DIMP by these dogs, and it is concluded that this compound produced no toxic effects at a dietary concentration of 3000 ppm or below, over the 90-day period of study.

DCPD

The test compound, DCPD Lot No. 040667, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

The test compound, DCPD W-761226, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

With the concurrence of the Project Officer, emphasis was placed on DIMP rather than DCPD with the result that no additional significant information was developed regarding DCPD. Previous findings are summarized below:

DCPD was absorbed after oral administration to mice, rats, and dogs. Peak plasma levels occurred in 2 hours in mice and dogs, and in 6 hours in rats. DCPD was widely distributed in all three species at 1 to 2 hours with the highest levels in urinary bladder, gall bladder and body fat in mice, in gall bladder and bile in dogs, and in body fat, adrenals and urinary bladder in rats. Excretion appeared to be primarily via the urine in all three species. About 85% of the administered radioactivity was accounted for in urine and feces within 24 hours. Urine from mice and dogs showed two radioactive components while rat urine also contained a third. All of these seemed to differ from DCPD on TLC, but none has yet been identified.

The test material was administered in the diet at doses of 80, 250 and 750 ppm to pregnant female rats on Days 6 through 15 of gestation. There were no changes in the dams or among the fetuses that indicated an adverse compound-related effect.

Two groups of 10 male and 20 female albino rats each (F0 generation) were given dicyclopentadiene (DCPD) in the diet at 80 or 750 ppm, with a similar group maintained as controls. The rats in each group were mated twice to produce the F1a and F1b litters.

The same number of F1b pups per sex per group were likewise mated to produce F2a and F2b pups, and the F2b animals were maintained to produce F3a and F3b litters. Analyses of the diet mixes indicated 87 and 92% of the desired concentrations were achieved, on the average, for the low- and high-dose levels, respectively.

For each generation in each group there were determined fertility indices, live-to-total pup ratios, mean litter sizes, pup survival indices and mean body weights at Day 4 post partum and at weaning. Gross necropsy observations were made of representative pups of all F_a litters, of the F_{3b} litters, and of the parent rats. Body weights and food consumption were determined for parent rats at various intervals, also.

It is concluded no deleterious effects on reproductive processes or general condition of the rats were produced by DCPD in this study. Likewise, no evidence of dose-related teratologic effect was seen.

The test material, Dicyclopentadiene, was administered by incorporation into the diet at concentrations of 100, 300, and 1000 ppm to Beagle dogs for 13 weeks. The animals were observed daily for general condition and behavior. Clinical pathological evaluations, including analysis of the clinical chemical constituents of serum, urine and hemograms, were performed at approximately monthly intervals. Tissues from the control and high dose dogs were compared histopathologically for differences. Based on the results obtained using these criteria it was concluded that treatment produced no significant toxicity with the possible exception of minor indications of intestinal distress expressed as vomiting and soft stools among dogs of the treated groups, especially the highest dose (1000 ppm).

FOREWORD

This report includes the results of microbial mutagenesis and demylination studies authorized under a predecessor contract (DAMD 17-75-C-5068).

In conducting the research described in this report, the investigator(s) adhered to the "Guide for Laboratory Animal Facilities and Care", as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

The method of euthanasia consisted of overdosage with carbon dioxide by inhalation in the case of group sacrifices or overdosage of pentobarbital sodium intraperitoneally or intravenously when one or a few individuals were sacrificed at a given time.

PART I - SECTION A
INTRODUCTION AND MATERIAL

DIMP

1. INTRODUCTION

In a continuation of an evaluation of the mammalian toxicity of DIMP, this compound has been studied by subchronic (90 day) administration to dogs, for reproductive and teratologic effects in rats, for neurotoxicity in chickens, for mutagenic effects in certain tester strains of salmonella and additional aspects of its metabolic fate have been investigated in mice, rats and dogs.

Earlier parts of the evaluation were reported in November 1976 under Contract No. DAMD 17-75-C-5068.

2. MATERIAL

DIMP (Diisopropylmethylphosphonate) was obtained as a custom synthesis from Richmond Organics, 7342 Forest Hill Avenue, Richmond, Virginia 23225. Three separate orders were placed and three shipments were received and designated as follows:

<u>Receipt Date</u>	<u>Quantity</u>	<u>LBi No.</u>
8/5/75	500 g	755A
12/15/75	500 g	776A
2/16/76	500 g	781A

DIMP was analyzed using an OV-17/Reoplex 400 column as described in the procedure for analysis of DIMP in water samples used by Shell Chemical Company and the Colorado Department of Health. DIMP had a retention time of 6.2 minutes. Two impurities were observed, one at 5.2 minutes and the other at 11.8 minutes. Content was calculated on a total peak area basis.

<u>LBi No.</u>	<u>DIMP</u>	<u>Impurity</u>	
		<u>#1</u>	<u>#2</u>
755A	95.2%	3.1%	1.7%
776A	89.6%	5.6%	4.8%
781A	88.0%	6.7%	5.3%

Because of poor water solubility, solutions were prepared for administration to animals by dissolving DIMP in polyethylene glycol 400 (PEG 400) "Carbowax" obtained from Fisher Scientific Company.

On January 8, 1977 an additional supply of DIMP was received from USAMBRDL. The bulk sample used for mammalian tests was provided by Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland, and purified by distillation through a 12 foot packed column under reduced pressure. The purity was estimated by gas chromatography to be approximately 96%, with impurities consisting most probably of triisopropyl phosphite and all five compounds obtainable from DIMP by interchange of isopropyl and methyl groups. A small sample of high purity (>99.9%) was prepared by reaction of methylphosphonodichloridate with isopropyl alcohol in basic medium for use in mutagenesis tests.

PART I - SECTION B
MICROBIAL MUTAGENESIS

DIMP

LBI PROJECT NO. 10734-01

SUMMARY

The test compound, DIMP USAMBRDL EPRD-1, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

The test compound, DIMP Lot AF-74, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: December 14, 1976
2. Description: Colorless liquid labelled USAMBRDL EPRD-1

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (diabasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. MATERIALS (Continued)

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^aConcentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

TABLE I-B-1

LITTON BIOMETRICS, INC.

4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: DIMP

B. SOLVENT: DMSO

C. TEST DATE: JAN. 3, 1977

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	ISSUE	8 EYE Q I A N I S P E H P L A I F											
			1A-1535		1A-1537		1A-1538		1A-98		1A-100		1A-100	
			1	2	1	2	1	2	1	2	1	2	1	2
NONACTIVATION														
SOLVENT CONTROL POSITIVE CONTROL** TEST COMPOUND	---	---	12	20	18	37	191	37	191	37	191	37	191	37
	---	---	>1000	789	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	---	---	21	18	26	35	188	35	188	35	188	35	188	35
	---	---	23	14	28	37	205	37	205	37	205	37	205	37
	---	---	21	13	28	27	163	27	163	27	163	27	163	27
0.10000 UL 1.00000 UL 5.00000 UL	---	---	14	11	29	32	207	32	207	32	207	32	207	32
	---	---	15	10	33	28	185	28	185	28	185	28	185	28
	---	---												
ACTIVATION														
SOLVENT CONTROL POSITIVE CONTROL*** TEST COMPOUND	RAT	LIVER	20	23	25	35	196	35	196	35	196	35	196	35
	RAT	LIVER	>1000	891	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	RAT	LIVER	12	18	13	27	202	27	202	27	202	27	202	27
	RAT	LIVER	24	16	24	37	235	37	235	37	235	37	235	37
	RAT	LIVER	19	20	28	43	241	43	241	43	241	43	241	43
0.00100 UL 0.01000 UL 1.00000 UL 5.00000 UL	RAT	LIVER	9	19	25	34	233	34	233	34	233	34	233	34
	RAT	LIVER	13	22	29	33	223	33	223	33	223	33	223	33
	RAT	LIVER												

* IBY. CONVERTANTS PER PLATE

** 1A-1535	MNNG	10 UG/PLATE	*** 1A-1535	ANTH	100 UG/PLATE
1A-1537	QH	10 UG/PLATE	1A-1537	ANQ	100 UG/PLATE
1A-1538	NF	100 UG/PLATE	1A-1538	AAF	100 UG/PLATE
1A-98	NF	100 UG/PLATE	1A-98	AAF	100 UG/PLATE
1A-100	MNNG	10 UG/PLATE	1A-100	ANTH	100 UG/PLATE
04	MNNG	10 UG/PLATE	04	DHNA	100 MICROMOLEC/PLATE
SOLVENT	DMSO	2.5 %/PLATE	SOLVENT	DMSO	2.5 %/PLATE

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 μ l to 5 μ l per plate.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.


C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

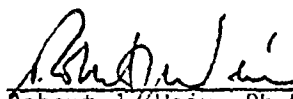
The test compound, DIMP USAMBRDL EPRD-1, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:


David J. Brusick, Ph.D.
Director
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1/26/77
Date

Reviewed by:


Robert J. Weir, Ph.D.
Vice President

1/27/77
Date

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Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a

6. EVALUATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

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To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: December 20, 1976
2. Description: Colorless liquid labelled Lot AF-74

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (diabasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^aConcentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the $9,000 \times g$ liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

TABLE I-B-2

LITTON BIOMEDICALS, INC.

4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: DIMP LOT AF-74

B. SOLVENT: DMSO

C. TEST DATE: JAN. 3, 1977

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	ISSUE	B. F. V. E. B. I. A. N. I. S. P. E. B. P. L. A. T. E.									
			IA-1535	IA-1537	IA-1538	IA-59	IA-100	IA-100	IA-100	IA-100	IA-100	IA-100
			1	2	1	2	1	2	1	2	1	2
NONACTIVATION												
SOLVENT CONTROL	---	---	12	24	20	22	112	24	112	24	112	24
POSITIVE CONTROL**	---	---	>1000	363	>1000	>1000	>1000	790	>1000	790	>1000	790
TEST COMPOUND												
0.00100 UL	---	---	24	34	15	25	125	12	125	12	125	12
0.01000 UL	---	---	35	32	20	34	107	23	107	23	107	23
0.10000 UL	---	---	31	26	16	26	101	22	101	22	101	22
1.00000 UL	---	---	25	27	19	29	80	27	80	27	80	27
5.00000 UL	---	---	27	24	24	31	50	3	50	3	50	3
ACTIVATION												
SOLVENT CONTROL	RAT	LIVER	20	33	27	37	108	125	108	125	108	125
POSITIVE CONTROL***	RAT	LIVER	>1000	285	>1000	>1000	>1000	255	>1000	255	>1000	255
TEST COMPOUND												
0.00100 UL	RAT	LIVER	23	41	9	38	114	68	114	68	114	68
0.01000 UL	RAT	LIVER	9	23	12	41	75	117	75	117	75	117
0.10000 UL	RAT	LIVER	10	20	16	21	140	125	140	125	140	125
1.00000 UL	RAT	LIVER	6	43	12	36	153	118	153	118	153	118
5.00000 UL	RAT	LIVER	25	22	8	38	116	113	116	113	116	113

* IBY. CONCENTRANTS PER PLATE

** TA-1535			** TA-1537			** TA-1538			** TA-100			** TA-100		
MINNG	10 UG/PLATE		MINNG	10 UG/PLATE		MINNG	10 UG/PLATE		MINNG	10 UG/PLATE		MINNG	10 UG/PLATE	
GH	10 UG/PLATE		GH	10 UG/PLATE		GH	10 UG/PLATE		GH	10 UG/PLATE		GH	10 UG/PLATE	
NF	100 UG/PLATE		NF	100 UG/PLATE		NF	100 UG/PLATE		NF	100 UG/PLATE		NF	100 UG/PLATE	
MF	100 UG/PLATE		MF	100 UG/PLATE		MF	100 UG/PLATE		MF	100 UG/PLATE		MF	100 UG/PLATE	
DMNG	10 UG/PLATE		DMNG	10 UG/PLATE		DMNG	10 UG/PLATE		DMNG	10 UG/PLATE		DMNG	10 UG/PLATE	
SOLVENT	2.5 %/PLATE		SOLVENT	2.5 %/PLATE		SOLVENT	2.5 %/PLATE		SOLVENT	2.5 %/PLATE		SOLVENT	2.5 %/PLATE	
DMSO			DMSO			DMSO			DMSO			DMSO		

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

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The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 μ l to 5 μ l per plate.

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The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

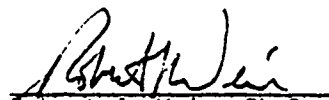
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Submitted by:


David J. Brusick, Ph.D.
Director
Department of Genetics

1/27/77
Date

Reviewed by:


Robert J. Weir, Ph.D.
Vice President

1/27/77
Date

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
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A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

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Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

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Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

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PART I - SECTION C

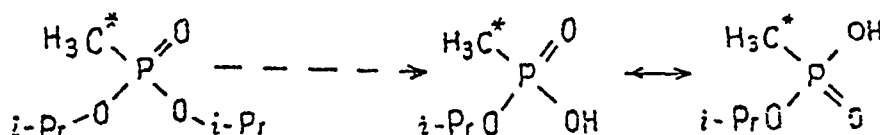
PHARMACOKINETICS AND METABOLISM

DIMP

LBI PROJECT NO. 10734-02

SUMMARY

Greater than 90% of the radioactivity of administered labelled diisopropyl methyl phosphonate (DIMP) to rats, mice and dogs was recovered in the urine. In all three species about 95% of recovered radioactivity comes from a single highly polar component. This component is identified as isopropyl-methyl-phosphonic acid (IMPA). The identification is based on comparing the metabolite with authentic IMPA by three different chromatographic techniques; namely, thin layer chromatography, ion chromatography and gas liquid chromatography coupled with mass spectrometry.



1. Objective:

The purpose of this study was to isolate and identify the major metabolite of diisopropyl-methyl ($^*\text{CH}_3$) phosphonate (^{14}C -DIMP) in the urine of rats, mice and dogs, following a single oral dose.

2. Material:

DIMP (Lot No. 922-057), labelled in the methyl position with ^{14}C , was synthesized by New England Nuclear Corporation, Boston Massachusetts. The specific activity was $3.07 \mu\text{Ci/mM}$ and the purity was greater than 99% as indicated by gas and thin-layer chromatography.

IMPA was supplied by Dr. Leon Schiff of DRDAR, Edgewood, Aberdeen Proving Grounds, Maryland. The compound was analyzed for purity by thin-layer chromatography using three different solvent systems. The results showed purity $> 97\%$.

The urine collected in the first phase of the study (1976) was used here for investigating the metabolites. The urine was stored frozen at -20°C . The urine was rechecked chromatographically and no significant changes in the initial pattern were found, indicating that no appreciable change has occurred over the storage period.

Rat and mouse 0-24 hours urine was pooled and used in this phase.
Dog 0-24 hours urine from 2 different animals was used interchangeably.

All solvents used were analytical grade reagents. TLC plates precoated with silica gel G, as well as cellulose, were obtained from Brinkman Instrument Inc, Westbury, New Jersey.

3. Experimental Design:

A. Extraction of radioactivity from urine:

(1) Extraction of DIMP from urine:

Two to five ml of 0-24 hour urine of all three species, as well as control urine spiked with ^{14}C -DIMP, were extracted with three equal volumes of chloroform.

(ii) Extraction of DIMP metabolites from urine:

In another experiment, urine from the three species was acidified with conc HCl (2 to 3 drops per ml) and extracted with 3 equal volumes of water saturated 1 Butanol.

(iii) Percentage distribution of radioactivity between two phases:

In both cases, after separating the two phases by centrifugation, 50 μl aliquots of each phase were placed in a scintillation vial with 20 ml of Hydromix (liquid scintillation cocktail for aqueous samples, Yorktown Research, New Jersey) and counted. The percentage distribution of radioactivity between the two phases was then calculated.

B. Thin Layer Chromatography:

Ten to thirty microliter samples containing 3000 to 5000 cpm were spotted on TLC silica gel G as well as cellulose plates. Several solvent systems were used for developing the TLC plates. The radioactive spots on the TLC plates (5 x 20 cm) were localized by scanning with a radiochromatogram scanner, (Model 7201, Packard Instrument Co.) at appropriate settings for time constant (10 seconds), linear range (300 cpm) and chart speed (1.0 cm/min). Radioactivity present in each peak area was quantitated by a disc integrator. Further localization was made by placing the plates on Kodak XR-1 x-ray film in an 8" x 10" cassette for 1 to 2 weeks. The films were developed and copies of the positive spots were made. In order to quantitate activity, the spots were scraped directly into scintillation vials containing 15-20 ml of scintillation fluid. The samples were counted and the radioactivity was calculated as percent in each zone.

C. Enzyme Hydrolysis:

To one ml of urine from each of the three species, 1 ml of 0.1M acetate buffer (pH 5.0) was added, as well as 0.1 ml of gluculase containing 176,133 units/ml β glucuronidase and 42,048 units sulphatase (Lot No. KN409A, Endo Laboratories, Garden City, New York). The mixtures were incubated for 24 hours at 37°C in a shaking water bath.

The hydrolyzed urine was then subjected to thin layer chromatography as described above. The chromatographic pattern was compared to non-hydrolyzed samples of the same urine.

D. Isolation & Purification:

One ml of 0-24 hour rat DIMP urine, as well as control rat urine, were each spread across a 20 x 20 cm, 2 mm thick silica gel plate, one inch from the base.

The plate was developed in 1-Butanol:Water:Acetic acid (5:4:1). (The solvents were thoroughly mixed in a separatory funnel, the phases were allowed to separate and the aqueous layer was discarded.)

The radioactivity was first visualized by radio-autography using Kodak XR-1 X-ray film. The radioactive zone was then localized and scraped off from the plate into a 50 ml centrifuge tube and extracted with 3 x 15 ml of methanol. All the methanol extracts were combined and centrifuged at 9000 rpm and filtered through a 0.45 micron millipore filter. The methanol was then taken to dryness at 40°C under a stream of nitrogen. An equivalent zone from the plate with the control urine was scraped, extracted, filtered and taken to dryness.

E. Preparing the methyl derivative of the major metabolite.

Approximately 2 millimoles (250 mg) of MNNG (N-methyl-N'-nitro-N-nitroso guanidine) were placed in the inside tube of the diazomethane generator (Aldrich) with half a milliliter of water to dissipate any heat generated. Ether (\approx 3 ml) was placed in the outside tube and the two parts were assembled with a butyl-O-ring and held with a pinch-type clamp. The lower part was immersed in an ice bath and about 0.6 ml of 5 N NaOH was injected dropwise through the silicone rubber septum by a syringe. The diazomethane thus formed was collected into the ether layer.

Two ml of diazomethane in ether prepared as above were added to each of three separate vials, one containing 2 microliters of neat IMPA, the second had the fractionated metabolite as described in section 'D', and the third had the fractionated control (section 'D').

The vials were capped and allowed to stand overnight. The ether was then removed under a stream of nitrogen and all 3 vials were reconstituted in 1.0 ml of methanol.

Gas Chromatography/Mass Spectrometry:

A Finnigan GC/MS model 4000 equipped with 6110 data system was used to acquire both EI & CI spectra for the methyl derivatives of IMPA, the urinary major metabolite and urine control. These were prepared as described in section E (Experimental Design).

A 6' x 2 mm I.D. glass column packed with 10% FFAP on 80/100 Chromosorb W AW was used. The other GLC conditions were set as follows: flow rate was held at 20 ml/min of He, the injector temperature was set at 170°C, the separator at 200°C and the transfer line at 210°C. The column temperature was held at 100°C for 2 minutes, then temperature programmed to 170°C at 5°C/min.

The mass spectrometer was tuned and calibrated with FC-43. The following conditions were used: electron energy - 70 ev, emission current - 250 mA, E.M. voltage - -1700 v, ion source temperature = 200°C in EI mode and 150°C at isobutane CI mode. Under computer control, the mass spectrometer was scanned repetitively from m/e 40 to 200 in EI mode and m/e 60 - 250 in CI mode at a rate of 2 sec/scan.

After the sample was injected to the gas chromatograph, the solvent was diverted for 110 sec. and the data acquisition was started at 120 sec.

Ion Chromatography

For the analysis of the major metabolite by ion chromatography, it was necessary to prepare a sample of non-radioactive metabolite. A rat weighing 225g was dosed with 1 ml of non-radiolabeled DIMP in PEG 400 (48.8 mg/ml). The rat was maintained in a Roth metabolism chamber with food and water ad lib. for a 24-hr period. A total of 5 ml of urine was collected.

A 0.25 ml aliquot (1.7 mg IMPA) of the urine was streaked across a TLC plate 1 inch from the bottom edge. On each end of the plate a marker (aliquot of a 24-hr urine specimen from a rat administered ¹⁴C-DIMP) was also spotted. The TLC parameters were:

Plate: 200 micron silica gel 60 F-254, 20 x 20 cm.

Solvent system: n-propanol/benzene/diethyl ether/ethanol/
2N NH₄OH (30/10/20/20/20)

Development: 107 mm from origin.

The two ends (sides - edges) of the plate containing the radioactive marker were removed with a glass cutter and scanned in a Packard Model 7201 Radiochromatogram Scanner. The Rf of the radio-labeled metabolite was determined to be 0.38.

The TLC plate was reassembled and the area at Rf 0.38 corresponding to the non-radiolabeled metabolite was scraped off. One-half of the material, containing approximately 850 μ g of suspected IMPA, was eluted by shaking with three 5.0 ml portions of deionized distilled water. The material was centrifuged after each shaking and the supernatants further clarified by passing through a 0.45 micron Millipore filter.

A control urine sample (0-24 hour rat urine) was processed similarly and the area corresponding to Rf of the metabolite was scraped and treated in the same way.

A third sample was scraped from a silica gel plate developed in the same solvent system but with nothing applied on it.

RESULTS AND DISCUSSION:

A. Extraction:

Table I summarizes the results of chloroform extraction experiment.

Table I

Percent ^{14}C Radioactivity Extracted into Chloroform From 0-24 Hour
Mouse, Rat, and Dog Urine After Administration of a Single
Oral Dose of 225 mg/kg of DIMP- ^{14}C

Sample	% ^{14}C Extracted In Chloroform	% ^{14}C Remaining In Urine	Total % ^{14}C Recovered
Chloroform Extract Control Urine Spiked With DIMP- ^{14}C	98.37	1.63	100.00
Chloroform Extract 0-24 Hour Mouse Urine	3.23	95.97	99.20
Chloroform Extract 0-24 Hour Rat Urine	2.80	96.30	99.10
Chloroform Extract 0-24 Hour Dog Urine	1.10	99.00	101.10

As shown in Table I, over 98% of the radioactive DIMP was extracted to the chloroform layer. However, the low percentage of radioactivity present in the chloroform extract of the 0-24 hr urine from each of the three species indicates that most of the DIMP was metabolized to more polar compound(s) and remained in the aqueous phase.

The results of the water saturated butanol extract (Table II) shows a marked similarity across the three species. It also reflects the highly polar nature of the metabolite.

Table II

Sample	% ¹⁴ C Extracted in water sat. 1-butanol	% ¹⁴ C Remaining in urine
Dog (0-24 hour urine)	88.7	11.3
Mouse (0-24 hour urine)	82.8	17.2
Rat (0-24 hour urine)	87.1	12.9

B. Fractionation of Radioactive components by TLC.

Distribution of Radioactivity:

Urine from rat, mouse and dog containing approximately 5000 cpm were each spotted on a 5 x 20 cm silica gel plate (0.25 mm thick). The plates were then developed in n-butanol: H₂O:acetic acid. Table III summarizes the findings of these experiments.

Table III

THIN LAYER ANALYSIS OF 24-HOUR URINE SAMPLES
FROM RAT, MOUSE AND DOG DOSED WITH ¹⁴C-DIMP

Species	Metabolite A (IMPA)		Metabolite B		Metabolite C		Metabolite D	
	Rf	%	Rf	%	Rf	%	Rf	%
Rat	0.12	93.2	0.31	2.7	0.56	2.6	0.65	1.5
Mouse	0.17	95.6	0.36	3.5	ND	ND	0.72	0.9
Dog	0.15	99.6	0.31	0.4	ND	ND	ND	ND

System: Silica Gel: n-butanol/water/acetic acid (50/40/10)

In this system, ¹⁴C-DIMP had an Rf of 0.60.

Again the results here stress the similarity across the species at least as far as the major metabolite is concerned.

Relation of major metabolite to IMPA

Further thin layer work was done using different solvent systems and different stationary phases. Urine from species containing approximately 5000 cpm were spotted on both 5 x 20 cm (cellulose and silica gel) plates at the origin, one inch from the base. Five μ l of 5% IMPA aqueous solution were spotted alongside the urine. The plates were then developed by two different solvent systems. The metabolite was localized by radioscanning. IMPA was localized by iodine vapor.

Table IV

TLC System	Dog		Mouse		Rat	
	$^{14}\text{C}(\text{R}_f)$	IMPA(R_f)	$^{14}\text{C}(\text{R}_f)$	IMPA(R_f)	$^{14}\text{C}(\text{R}_f)$	IMPA(R_f)
Cellulose plate Solvent system A	0.42	0.45	0.40	0.42	0.42	0.47
Silica gel plate Solvent system B	0.29	0.32	0.30	0.34	0.33	0.34

Solvent system A: n-butanol: n-propanol: 1N NH_4OH (3:1:1)

Solvent system B: benzene: l-propanol: ethanol: ether: 2N NH_4OH (1:3:2:2:2)

Here again the R_f values show the species similarity and that the metabolite is identifiable as IMPA.

c. Enzyme hydrolysis:

An experiment was set up as described in Section C to determine if the metabolites were conjugated to glucuronic acid. The urine, before and after incubation was spotted on silica gel G and developed in n-butanol: H_2O : acetic acid (5:4:1). No difference in the pattern of radioactivity distribution was found. Extraction with CHCl_3 before and after enzyme incubation showed no appreciable difference in percent distribution of radioactivity.

This is taken as good evidence that glucuronic acid and sulphate conjugation do not take place in DIMP metabolism.

d. Ion Chromatography:

The samples prepared as described above (experimental design Section E) were sent to Dr. Leon J. Schiff of DRDAR-CLB-CA, Bldg. 3220, Edgewood Area, Aberdeen Proving Ground, Md. 21010, for ion chromatographic analysis.

The ion chromatograms obtained are shown in figures 1 thru 4. Fig. 1 is the ion chromatogram of 5.0 ppm IMPA standard in 0.005M sodium borate buffer; fig. 2 is that of control urine silica gel extract; fig. 3 is blank silica gel extract and fig. 4 is the ion chromatogram of DIMP 0-24 hrs urine metabolite, silica gel TLC extract.

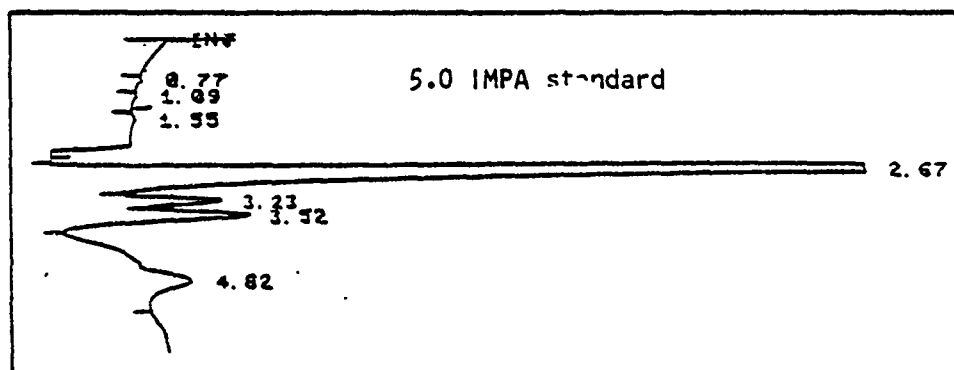


Figure 1

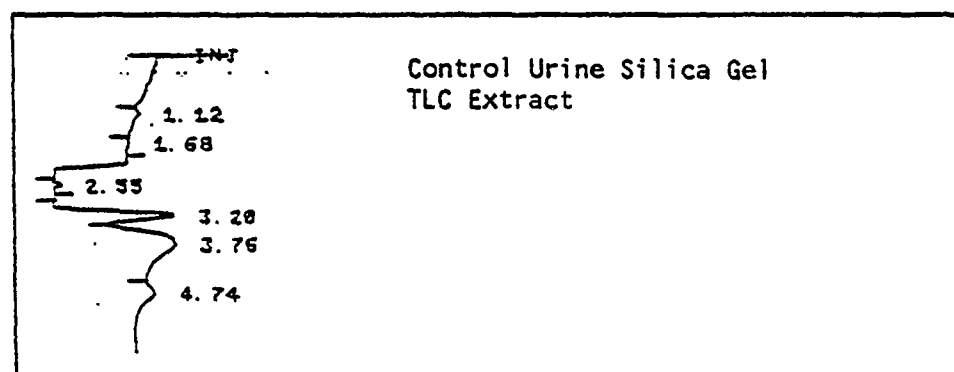


Figure 2

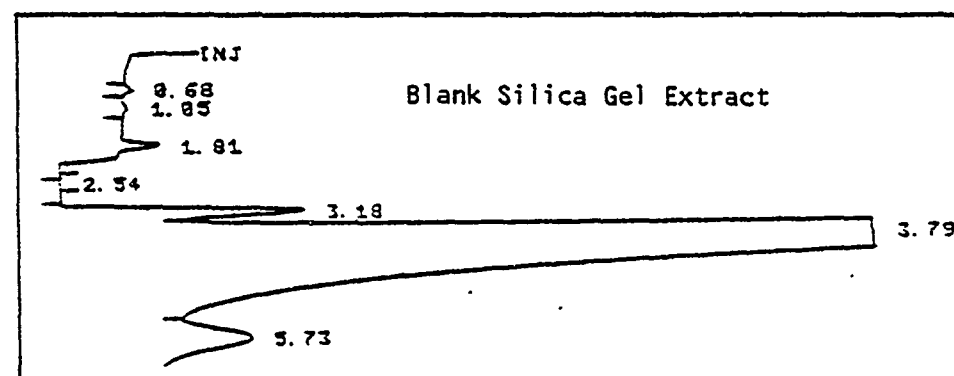


Figure 3

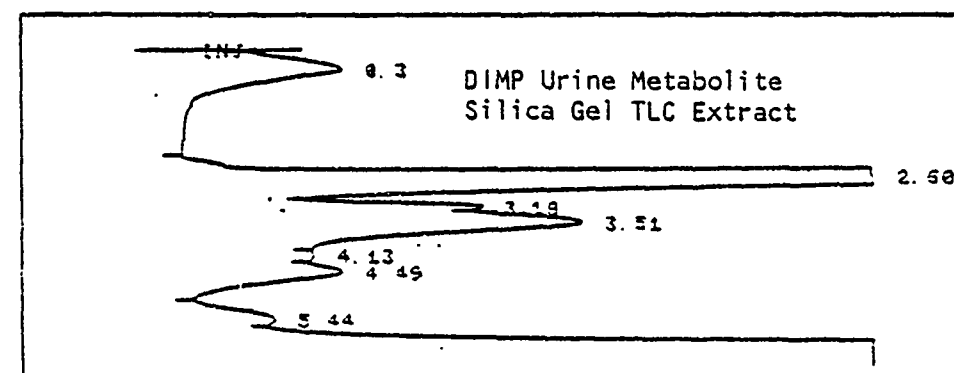
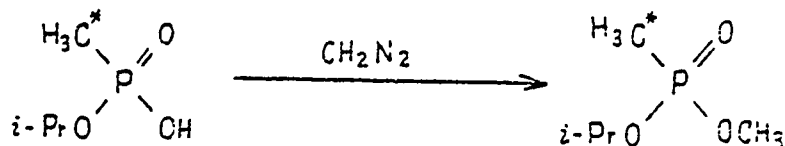


Figure 4

It is evident that only the DIMP metabolite sample showed a peak which matched the retention time of IMPA standard at 2.6-2.8 min region. This provides more evidence that the major metabolite isolated from rat urine is IMPA.

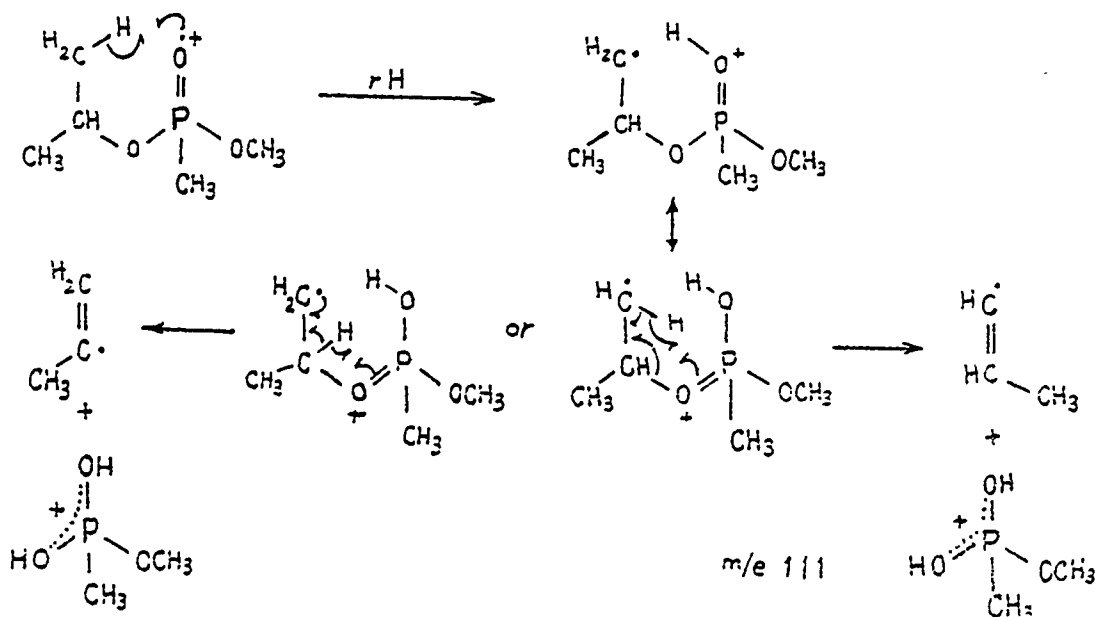
e. Gas Chromatography/Mass Spectrometry Identification of Major Metabolite

Attempts had been made to analyze intact IMPA gas chromatographically without any success. This may be due to its high polarity and low volatility which were overcome by forming a methyl derivative with diazomethane.



IMPA standard, fractionated rat urine extract and fractionated urine control extract were methylated under the same conditions as described in Section D. All of them were submitted to both EI and isobutane CI GC/MS.

Under electron impact conditions, comparison of the total ion current (TIC) trace obtained from the methylated major metabolite isolated from rat urine (Fig. 5) with IMPA methyl ester (Fig. 6) and with methylated urine control (Fig. 7) clearly indicated that the peak with a retention time of 8.3 min (scan no. 197) from the methylated major metabolite was isopropyl methyl methyl phosphonate (IMMPA). The same peak was absent in the control urine. The EI mass spectrum (Fig. 8) taken at that peak was in good agreement with that of the IMMPA standard (Fig. 9). Although the molecular ion (m/e 152) intensity in both cases was quite low, the same fragmentation pattern and other characteristic ions confirmed the identity of the major methylated metabolite as IMPA methyl ester. The ions at m/e 137 and m/e 93 correspond to $(M - \text{CH}_3)^+$ and $M - \text{OCH}(\text{CH}_3)_2^+$ respectively. The second strongest ions at m/e 111 may arise from double hydrogen rearrangement ("McLafferty + 1" rearrangement):



409905; 5 UL METABOLITE EXTRACT DERIVATIZED
196 - BKG #190

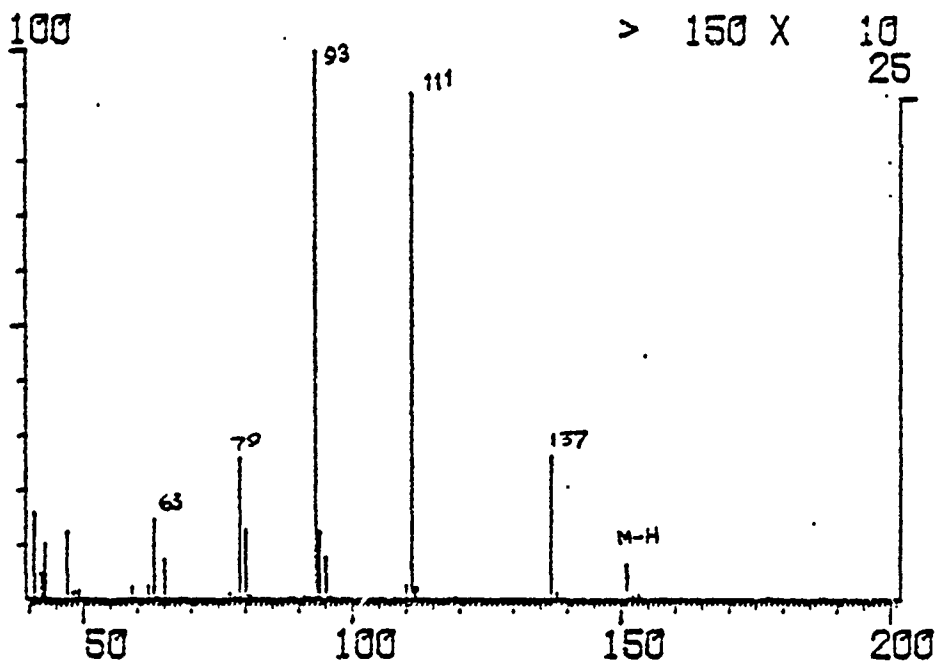


Figure 8

IMPA METHYL ESTER; STANDARD; EI GC/MS
197 - BKG #206

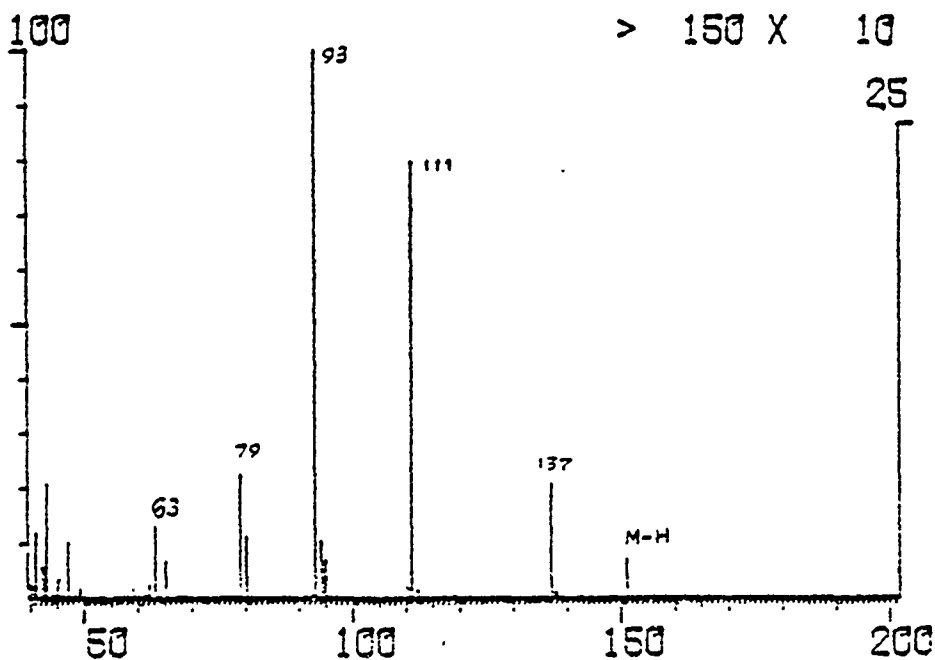


Figure 9

409905: 5 UL METABOLITE EXTRACT DERIVATIZED
EI GC/MS

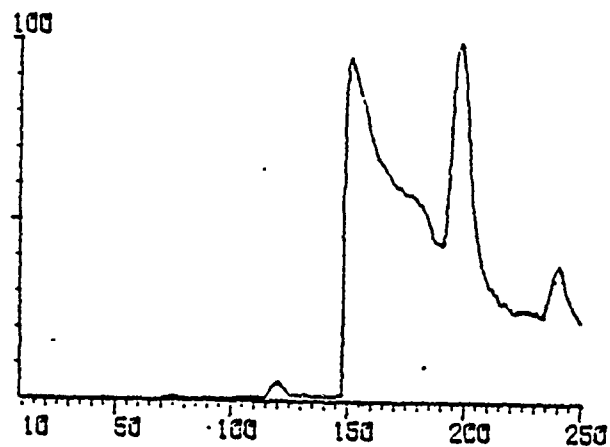


Fig. 5

409904: IMPA METHYL ESTER; STD.; EI GC/MS

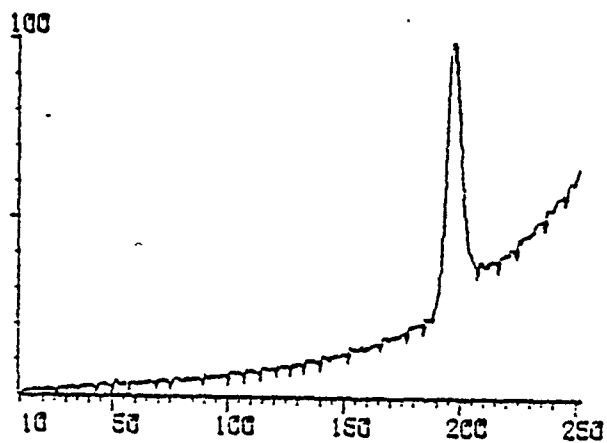


Fig. 6

409906: 5 UL DERIVATIZED CONTROL URINE
EI GC/MS

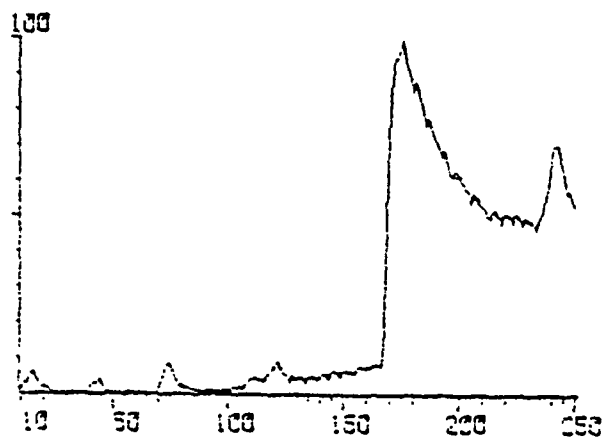


Fig. 7

412902:ME DERIV OF METABOLITE CI (ISO-B)
- BKG=189

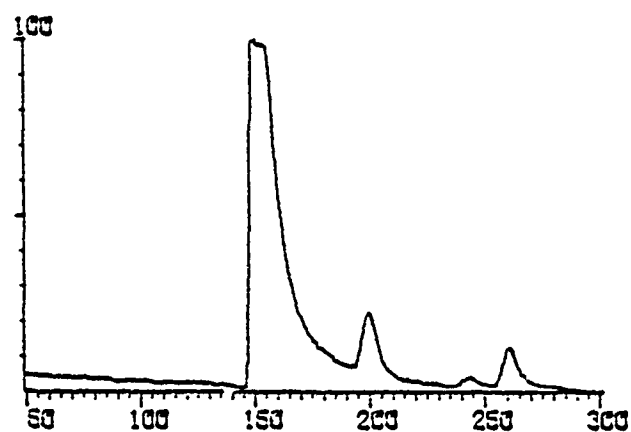


Fig. 10

412901: CI (ISO-B) ME DERIV OF IMPA → 2NG
- BKG=189

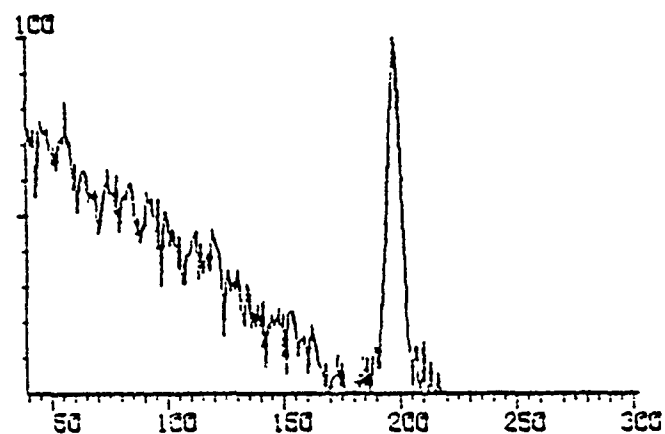


Fig. 11

412903: ME DERIV OF CONTROL URINE CI (ISO-B)

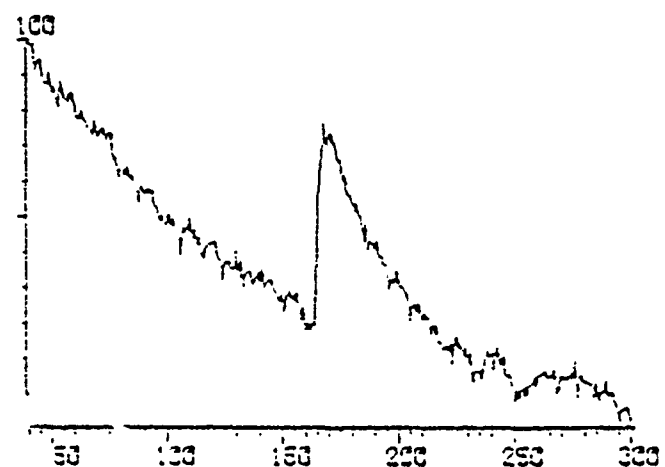


Fig. 12

412901; CI (ISO-B) ME DERIV OF IMPA -- 2NG
197 - BKG#189

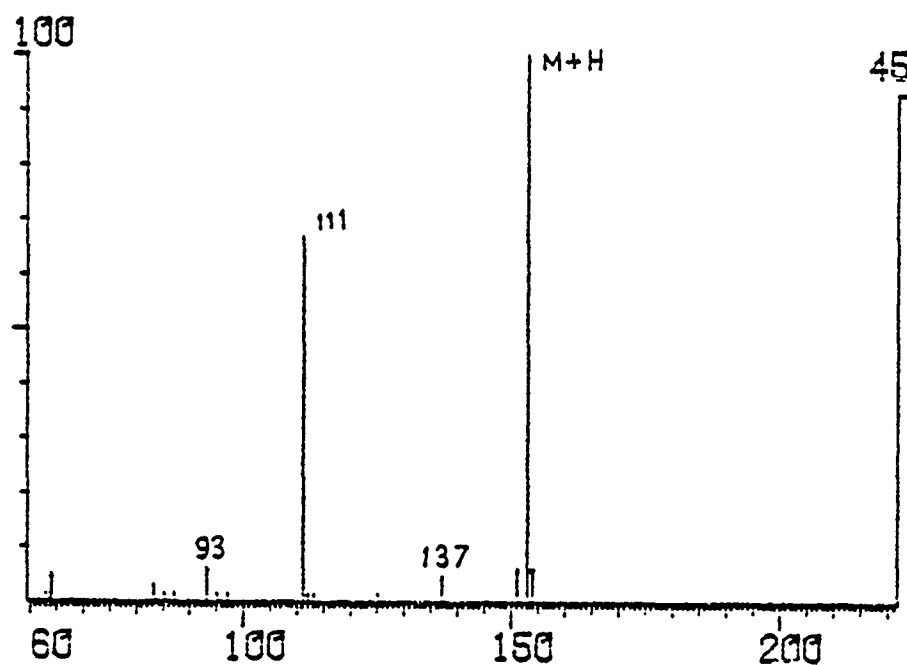


Figure 13

412902; ME DERIV OF METABOLITE CF (1 C-
199 - BKG#189

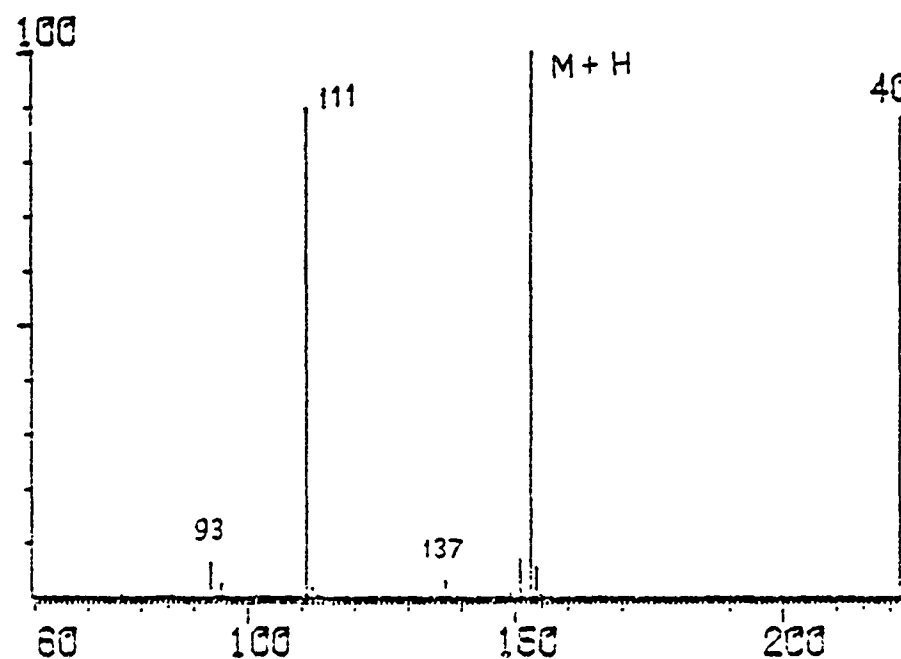
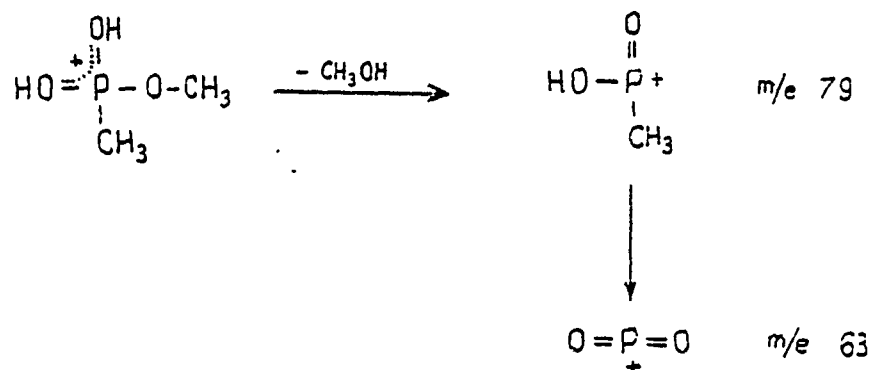


Figure 14

The ions at m/e 79 may be attributed up to a loss of CH₃OH from the m/e 111 ions:



Further evidence for the identity of the major metabolite as IMPA was provided by isobutane CI GC/MS. The super-imposition of the retention time of the peak from the methylated major metabolite (Fig. 10) with that from the IMPA (Fig. 11) was in excellent agreement with the EI GC/MS data. Once again, that peak was totally absent in the control urine (Fig. 12). CI mass spectrum from the methylated major metabolite (Fig. 13) and that from IMPA methyl ester (Fig. 14) were almost identical. The quasimolecular ion m/e 153, (M + H)⁺, in both spectra unambiguously confirmed it was IMPA methyl ester.

The ions at m/e 137 (M + H = CH₄)⁺ and m/e 111 share the same structure as the corresponding ions formed in the EI mode.

5. Conclusion

Based on the R_f value on two different TLC plates and three different developing solvent systems, ion chromatograms, GLC retention time as well as EI and isobutane CI mass spectra, it is evident that the major metabolite isolated from the urine of rats dosed with DIMP can be identified as IMPA (isopropyl methyl phosphonic acid).

PART I - SECTION D

TERATOLOGY IN RATS

DIMP

LBI PROJECT NO. 10734-04

SUMMARY

The test material was administered in the diet at doses of 100, 300 and 3000 ppm to pregnant female rats on days 6 through 15 of gestation. There were no changes in the dams or among the fetuses that indicated an adverse compound-related effect.

1. OBJECTIVE

The objective of this study was to investigate the effect of the test material on fetuses during the period of organogenesis when administered to the pregnant rat.

2. MATERIAL

Refer to Part I - Section A.

3. EXPERIMENTAL DESIGN

Female rats [CRL:COBS CD (SD) BR] were obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for one week. At that time, each female was paired with a sexually mature male of the same strain from the same supplier. The females were examined daily for the presence of a copulatory plug. The presence of such a plug was taken as evidence of mating and designated as day 0 of gestation. The female rats were 11 weeks of age at the time of the first dose (March 3, 1977). Mated female rats were assigned sequentially to treatment groups and identified by cage cards as indicated on the following page.

3. EXPERIMENTAL DESIGN (Continued)

<u>Group No.</u>	<u>Female Rat Numbers</u>	<u>Dose in Diet (ppm)</u>
1	4720 - 4739	0 (Control)
2	4740 - 4759	100
3	4760 - 4779	300
4	4780 - 4799	3000

These doses were approved by Dr. E. Ross Hart of Litton Bionetics, Inc., based on previous studies.

The female rats were individually housed in wire cages in a temperature-controlled animal room with artificial illumination automatically controlled to provide a 12-hour light cycle. No other species were housed in the animal room during the course of the study. No other chemicals were under concurrent investigation in the animal room. The appropriate diets and fresh water were provided ad libitum. The test material was incorporated into the basal diet (Purina Laboratory Chow Meal) on gestation days 6 through 15 so as to provide the dose levels indicated previously. The test material (1, 3 or 30 g) was suspended in 100 ml of polyethylene glycol 400 and blended with 10 kg of the basal diet in a twin shell blender for 15 minutes. The control diet contained 100 ml of PEG 400 per 10 kg of meal.

The mated female rats were weighed on days 0, 6, 16 and 20 of gestation. Food consumption was measured during the periods 0-6, 6-16 and 16-20 days of gestation. The female rats were observed daily for changes in general appearance, behavior and condition.

On day 20 of gestation, the adult female rats were anesthetized with chloroform and the visceral and thoracic organs examined. The uterus was removed and opened. The number of implantation sites and their placement in the uterine horns, live and dead fetuses, and resorption sites were recorded. The fetuses were removed, examined externally for abnormalities, weighed and the crown to rump length was measured.

One third of the fetuses of each litter were fixed in Bouin's fluid. These were later examined for changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses of each litter were examined for skeletal abnormalities following staining with Alizarin Red S.

The uterus and ovaries from the adult females were preserved in 10% formalin for possible future examination.

4. RESULTS

No deaths occurred among the adult female rats. Except for female rat number 4735 (control), these animals were normal in appearance throughout the study. Rat number 4735 delivered a litter of pups on her calculated day 19 of gestation. Because the pups appeared to be full term, the assignment of day 0 of gestation was judged to be erroneous, possibly due to the retention of the copulatory plug by the female. Although the data on this litter was included in Table 2 of the Appendix, it was not used in the calculations or summary.

Mean body weight and food consumption, as shown in the Appendix on Table 1, indicated no marked difference between treated and control pregnant rats ($p < 0.05$ Dunnett's t-test, Americal Statistical Assn., 50: 1096-1121, 1955).

Based on the observations of the uterine contents obtained on day 20 of gestation, the test material did not produce any effect. These data have been summarized in Table A and the details have been tabulated in Table 2 included in the Appendix. Although a 2×2 contingency with Yate's correction (Hollander and Wolfe) analysis of the total number of implantation sites in both the right and left horns in the 300 ppm dose level shows a significant decrease from the control group, a Wilcoxon Rank Sum analysis (Snedecor and Cochran Statistical Methods, Iowa State Press, Ames, Iowa, pp. 215-223, 1974) did not indicate a significant difference ($p < 0.05$) and this effect was judged to be a statistical artifact. Examination of the internal organs of the females revealed no abnormalities except for an ovarian cyst in female rat number 4774 (300 ppm). This change was not judged to be related to treatment.

Examination of the offspring at delivery revealed subcutaneous hematomas on two fetuses of the control group (4729 and 4738) and on one fetus of the 3000 ppm dose group (4797). In addition, two fetuses of female number 4769 (300 ppm) were dead, although, apparently at a normal stage of development on day 20 of gestation; and one fetus of female rat number 4740 (100 ppm) had a short lower jaw. This last abnormality was also observed at examination of cleared specimens as a malformation of the maxilla and mandible.

TABLE I-D-3

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE A

SUMMARY OF REPRODUCTION PERFORMANCE

	DOSE (ppm)			
	<u>0</u>	<u>100</u>	<u>300</u>	<u>3000</u>
Pregnancy Ratio (Pregnant/Bred)	14/20	14/20	11/20	15/20
Implantation Sites (Left Horn/Right Horn)	86/67	78/90	50 ^b /68 ^b	91/91
Resorptions/Live Fetuses	13/140	16/152	7/110	9/173
Average Fetal Weight (G) ^a	3.7	3.9	3.8	3.9
Average Fetal Length (cm) ^a	3.1	3.1	3.1	3.1
Mean Live Litter Size (pups)	11	11	10	12

^aBased on average of litter means

^b $p < 0.05$ 2 x 2 contingency table with Yate's correction; not significant $p > 0.05$
Wilcoxon Rank Sum (LSA)

4. RESULTS (Continued)

Examination of the Bouin's fixed specimens revealed one fetus of female rat number 4732 (control) with posterior displacement of the left kidney. There were no other changes observed. The sex and number of fetuses examined for soft tissue changes were as follows:

<u>Treatment</u>	<u>Males</u>	<u>Females</u>
0 (Control)	26	27
100	21	29
300	17	20
3000	30	28

The results of the skeletal examination of the cleared and stained fetuses have been detailed in Appendix Table 3. Most of the changes noted, while not strictly normal, are frequently observed in 21 day old rat fetuses of this strain and source in our laboratory.

Those findings not commonly encountered have been so indicated in Table 3. The results have been summarized below:

<u>Dose</u>	<u>Number Examined</u>	<u>Number Normal</u>	<u>Number with Common Skeletal Variations</u>	<u>Number with Unusual Changes</u>
Control	100	73	27	0
100 ppm	102	69	33	2
300 ppm	75	58	15	4
3000 ppm	114	67	45*	3

* ($p < 0.05$ 2 x 2 contingency table)

There was a slight increase in the ratio of abnormal/normal fetuses at the high level as compared to the control. However, analysis of these data using Wilcoxon Rank Sum analysis which employs the litter as the basic unit for comparison, did not indicate a statistically significant effect. Furthermore, the nature of the changes observed did not suggest a specific area of involvement. Therefore, the changes observed were not judged to indicate a drug-induced teratogenic effect at this dose.

5. CONCLUSION

Administration of the test material to female rats by incorporation into the diet at 100, 300 and 3000 ppm produced no effect on the pregnant dams. There was no evidence of compound-induced terata, variation in sex ratio, embryo toxicity or inhibition of fetal growth and development.

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Robert J. Weir, Ph.D.
Vice President

TABLE I-D-4

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 1

BODY WEIGHT AND FOOD CONSUMPTION OF PREGNANT RATS

DOSE (ppm)	MEAN BODY WEIGHTS IN GRAMS ^a				MEAN DAILY FOOD CONSUMPTION IN GRAMS ^a			
	DAY 0	DAY 6	DAY 16	DAY 20	DAY 0-6	DAY 6-16	DAY 16-20	
0 (Control)	Mean	214	240	281	20	18	25	
	SD	17	19	22	7	1	5	
	SE	4.6	5.4	6.1	2.2	0.6	1.3	
	n	13	13	13	10	5	13	
100	Mean	219	245	299	22	20	23	
	SD	17	16	27	9	2	4	
	SE	4.5	4.2	7.3	2.3	0.5	1.0	
	n	14	14	14	13	11	14	

^aCalculations do not include non-pregnant females

TABLE I-D-4 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 1 (Continued)

BODY WEIGHT AND FOOD CONSUMPTION OF PREGNANT RATS

DOSE (ppm)		MEAN BODY WEIGHTS IN GRAMS ^a			MEAN DAILY FOOD CONSUMPTION IN GRAMS ^a		
		DAY 0	DAY 6	DAY 16	DAY 0-6	DAY 6-16	DAY 16-20
300	Mean	217	242	286	18	19	25
	SD	19	19	25	2	3	4
	SE	5.6	5.8	7.5	0.7	1.0	1.1
	n	11	11	11	11	7	11
3000	Mean	217	247	295	19	21	26
	SD	15	13	19	1	2	3
	SE	3.9	3.5	4.8	0.3	0.8	0.7
	n	15	15	15	15	8	15

^aCalculations do not include non-pregnant females

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE I-D-5

TABLE 2

OBSERVATIONS AT CAESAREAN SECTION

DOSE - Control (0 ppm)

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES	
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (gram)
4720	0	0	0	0	0	---
4721	0	0	0	0	0	---
4722	3	5	2	0	6	3.7
4723	4	5	3	0	6	3.9
4724	0	0	0	0	0	---
4725	8	6	1	0	13	3.7
4726	5	7	0	0	12	4.0
4727	3	2	1	0	4	3.5
4728	9	6	2	0	13	3.6
4729	10	7	0	0	17	3.6
4730	7	5	1	0	11	3.5
4731	0	0	0	0	0	---
4732	10	6	1	0	15	3.6
4733	0	0	0	0	0	---
4734	0	0	0	0	0	---
4735+	9	5	1	0	13	5.9
4736	8	6	0	0	14	3.7
4737	11	4	1	0	14	3.7
4738	8	6	1	0	13	3.6
4739	0	2	0	0	2	4.5
Total	86	67	13	0	140	
Mean*					11	3.7
SD					5	0.3
SE					1.3	0.07
n					13	13

*Mean, SD and SE do not include non-pregnant females

+Delivered prior to sacrifice (day 19), not included in Mean, SE and SE

TABLE I-D-5 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 2 (Continued)

OBSERVATIONS AT CAESAREAN SECTION

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES		MEAN LENGTH (cm)
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (gram)	
4740	2	0	1	0	1	3.4	3.0
4741	0	0	0	0	0	---	---
4742	0	0	0	0	0	---	---
4743	0	0	0	0	0	---	---
4744	12	2	0	0	14	3.9	3.2
4745	0	0	0	0	0	---	---
4746	0	0	0	0	0	---	---
4747	4	11	0	0	15	3.3	2.9
4748	1	0	0	0	1	4.2	3.5
4749	2	10	0	0	12	3.4	3.0
4750	7	9	1	0	15	3.6	3.2
4751	9	5	1	0	13	3.5	3.1
4752	5	6	7	0	4	3.7	3.2
4753	0	7	1	0	6	4.1	3.1
4754	8	7	2	0	13	4.3	3.1
4755	0	0	0	0	0	---	---
4756	5	9	0	0	14	3.6	3.1
4757	9	6	1	0	14	4.1	2.9
4758	6	8	1	0	13	3.7	3.2
4759	8	10	1	0	17	5.4	3.4
Total	78	90	16	0	152		
Mean*					11	3.9	3.1
SD					5	0.5	0.2
SE					1.4	0.15	0.05
n					14	14	14

*Mean, SD and SE do not include non-pregnant females

TABLE I-D-5 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 2 (Continued)

OBSERVATIONS AT CAESAREAN SECTION

DOSE - 300 ppm

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES		
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (gram)	MEAN LENGTH (cm)
4760	0	0	0	0	0	---	---
4761	0	0	0	0	0	---	---
4762	0	0	0	0	0	---	---
4763	2	5	0	0	7	4.0	2.9
4764	6	8	1	0	13	3.1	2.9
4765	4	0	0	0	5	3.8	3.0
4766	6	6	0	0	12	3.4	2.9
4767	1	6	0	0	7	3.4	3.0
4768	0	0	0	0	0	---	---
4769	1	10	1	2	8	3.6	3.1
4770	0	0	0	0	0	---	---
4771	0	0	0	0	0	---	---
4772	0	0	0	0	0	---	---
4773	4	9	3	0	10	4.6	3.2
4774	0	0	0	0	0	---	---
4775	11	5	1	0	15	3.3	3.1
4776	3	3	0	0	6	4.4	3.2
4777	4	8	1	0	11	5.0	3.3
4778	0	0	0	0	0	---	---
4779	8	8	0	0	16	3.6	3.0
Total	50	68	7	2	110		
Mean*					10	3.8	3.1
SD					4	0.6	0.1
SE					1.1	0.18	0.04
n					11	11	11

*Mean, SD and SE do not include non-pregnant females

TABLE I-D-5 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 2 (Continued)

OBSERVATIONS AT CAESAREAN SECTION

DOSE - 3000 ppm

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES		
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (gram)	MEAN LENGTH (cm)
4780	1	2	0	0	3	4.2	3.3
4781	0	0	0	0	0	---	---
4782	8	6	0	0	14	4.0	3.1
4783	0	0	0	0	0	---	---
4784	4	11	0	0	15	3.5	3.0
4785	6	6	1	0	11	4.1	3.1
4786	2	0	0	0	2	4.5	3.3
4787	8	5	2	0	11	3.7	3.2
4788	8	6	0	0	14	3.8	3.2
4789	0	0	0	0	0	---	---
4790	8	5	1	0	12	3.7	3.1
4791	0	0	0	0	0	---	---
4792	0	7	0	0	7	4.3	3.2
4793	8	5	0	0	13	3.8	2.9
4794	7	5	0	0	12	4.3	3.1
4795	0	0	0	0	0	---	---
4796	11	5	1	0	15	3.8	3.1
4797	9	9	2	0	16	3.5	3.1
4798	6	10	1	0	15	3.7	3.1
4799	5	9	1	0	13	3.4	3.0
Total	91	91	9	0	173		
Mean*					12	3.9	3.1
SD					4	0.3	0.1
SE					1.1	0.09	0.03
n					15	15	15

*Mean, SD and SE do not include non-pregnant females

TABLE 3

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - CONTROL

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4722	4	4 No visible skeletal abnormalities.
4723	4	3 No visible skeletal abnormalities. 1 Unilateral rib 14.
4725	9	7 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone.
4726	8	6 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone, reduced ossification of hyoid bone. 1 Bilateral rib 14.
4727	2	1 No visible skeletal abnormalities. 1 Non-fused thoracic vertebral centra.
4728	8	7 No visible skeletal abnormalities. 1 Unilateral rib 14.
4729	11	6 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, hyoid not ossified, reduced ossification of parietal bone. 1 Reduced ossification of interparietal bone. 2 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, hyoid not ossified. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone.

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE I-D-6 (Continued)

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE- CONTROL

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4730	7	5 No visible skeletal abnormalities. 1 Hyoid not ossified. 1 Unilateral rib 14.
4732	10	6 No visible skeletal abnormalities. 1 Hyoid not ossified, reduced ossification of interparietal bone, reduced ossification of supraoccipital bone. 1 Reduced ossification of interparietal bone. 1 Reduced ossification of hyoid bone. 1 Reduced ossification of interparietal bone, reduced ossification of hyoid bone, unilateral rib 14.
4735	9	8 No visible skeletal abnormalities. 1 Bilateral rib 14.
4736	9	9 No visible skeletal abnormalities.
4737	9	7 No visible skeletal abnormalities. 2 Reduced ossification of right rib 13.
4738	9	4 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone, reduced ossification of hyoid bone. 1 Hyoid not ossified. 1 Non-fused thoracic vertebral centra. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone.
4739	1	1 Reduced ossification of left rib 13.

TABLE I-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE- 100 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4740	1	*1 Maxilla malformed, mandible malformed, uni- lateral rib 14.
4744	9	2 No visible skeletal abnormalities. 3 Bilateral rib 14. 1 Unilateral rib 14. 2 Reduced ossification of interparietal bone, reduced ossification of hyoid bone. 1 Reduced ossification of hyoid bone.
4747	10	9 No visible skeletal abnormalities. 1 Bilateral rib 14.
4748	1	1 No visible skeletal abnormalities.
4749	8	6 No visible skeletal abnormalities. 1 Unilateral rib 14. 1 Bilateral rib 14.
4750	10	4 No visible skeletal abnormalities. 1 Hyoid not ossified. 2 Reduced ossification of interparietal bone, reduced ossification of hyoid bone. 1 Reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. *1 Reduced ossification of supraoccipital bone, reduced ossification of sternbrae, reduced ossification of pübes.
4751	8	7 No visible skeletal abnormalities. 1 Bilateral rib 14.
4752	3	2 No visible skeletal abnormalities. 1 Unilateral rib 14.
4753	4	4 No visible skeletal abnormalities.

*Not commonly encountered.

TABLE I-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 100 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4754	8	7 No visible skeletal abnormalities. 1 Bilateral rib 14.
4756	11	8 No visible skeletal abnormalities. 2 Unilateral rib 14. 1 Reduced ossification of hyoid bone.
4757	9	4 No visible skeletal abnormalities. 1 Unilateral rib 14. 1 Reduced ossification of hyoid bone, uni- lateral rib 14. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. 1 Hyoid not ossified. 1 Reduced ossification of hyoid bone.
4758	9	5 No visible skeletal abnormalities. 1 Hyoid not ossified. 1 Hyoid not ossified, unilateral rib 14. 1 Reduced ossification of hyoid bone. 1 Hyoid not ossified, reduced ossification of supraoccipital bone.
4759	11	10 No visible skeletal abnormalities. 1 Unilateral rib 14.

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE I-D-6 (Continued)

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 300 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4763	4	3 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone.
4764	9	6 No visible skeletal abnormalities. 2 Non-fused thoracic vertebral centra. *1 Reduced ossification of interparietal bone, hyoid not ossified, reduced ossification of sacral vertebral arches, reduced ossification of sternbrae, wavy ribs (right side), distal phalanges of hind extremities not ossified.
4765	3	1 Non-fused thoracic vertebral centra. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. *1 Reduced ossification of hyoid bone, reduced ossification of right ischium.
4766	8	7 No visible skeletal abnormalities. 1 Reduced ossification of hyoid bone.
4767	5	2 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone. *1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of parietal bone, unilateral rib 14, reduced ossification of hyoid bone. *1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of parietal bone, hyoid not ossified, reduced ossification of right ribs, wavy ribs (right side).
4769	6	6 No visible skeletal abnormalities.
4773	7	7 No visible skeletal abnormalities.
4775	4	3 No visible skeletal abnormalities. 1 Reduced ossification of hyoid bone.
4776	10	9 No visible skeletal abnormalities. 1 Bilateral rib 14.

*Not commonly encountered.

TABLE I-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 300 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4777	8	7 No visible skeletal abnormalities. 1 Unilateral rib 14.
4779	11	8 No visible skeletal abnormalities. 2 Bilateral rib 14. 1 Unilateral rib 14.

TABLE I-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE- 3000 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4780	2	2 No visible skeletal abnormalities.
4782	10	5 No visible skeletal abnormalities. 1 Reduced ossification of hyoid bone. 1 Non-fused thoracic vertebral centra. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone, reduced ossification of right rib 10. 1 Reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. *1 Reduced ossification of supraoccipital bone, reduced ossification of hyoid bone, reduced ossification of parietal bone, reduced ossification of interparietal bone.
4784	10	5 No visible skeletal abnormalities. 4 Unilateral rib 14. 1 Bilateral rib 14.
4785	7	3 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone, unilateral rib 14. 1 Reduced ossification of interparietal bone, reduced ossification of hyoid bone, non-fused thoracic vertebral centra. 1 Unilateral rib 14. 1 Reduced ossification of interparietal bone.
4786	1	1 Bilateral rib 14.
4787	7	6 No visible skeletal abnormalities. 1 Unilateral rib 14.
4788	9	6 No visible skeletal abnormalities. 2 Reduced ossification of hyoid bone. 1 Reduced ossification of hyoid bone, bilateral rib 14.

*Not commonly encountered.

TABLE I-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 3000 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4790	8	<ul style="list-style-type: none"> 1 No visible skeletal abnormalities. 1 Reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. 1 Reduced ossification of hyoid bone. 1 Reduced ossification of hyoid bone, unilateral rib 14. 1 Reduced ossification of supraoccipital bone, hyoid not ossified, unilateral rib 14. 1 Reduced ossification of interparietal bone. *1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone, reduced ossification of parietal bone, reduced ossification of maxilla, reduced ossification of sacral vertebral arches. 1 Reduced ossification of hyoid bone, bilateral rib 14.
4792	4	<ul style="list-style-type: none"> 3 No visible skeletal abnormalities. 1 Reduced ossification of interparietal bone.
4793	9	<ul style="list-style-type: none"> 5 No visible skeletal abnormalities. 1 Unilateral rib 14. 1 Bilateral rib 14. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone. 1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone.
4794	8	8 No visible skeletal abnormalities.
4796	10	<ul style="list-style-type: none"> 6 No visible skeletal abnormalities. 2 Reduced ossification of hyoid bone. 1 Reduced ossification of interparietal bone. 1 Reduced ossification of interparietal bone, reduced ossification of hyoid bone.

*Not commonly encountered

TABLE I-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 9004

TABLE 3 (Continued)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 3000 ppm

<u>FEMALE NUMBER</u>	<u>NUMBER OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
4797	10	3 No visible skeletal abnormalities. 2 Reduced ossification of interparietal bone, reduced ossification of hyoid bone. 2 Hyoid not ossified. 1 Reduced ossification of hyoid bone. 1 Reduced ossification of supraoccipital bone, reduced ossification of hyoid bone. 1 Reduced ossification of hyoid bone, uni- lateral rib 14.
4798	10	5 No visible skeletal abnormalities. 1 Hyoid not ossified. 2 Reduced ossification of hyoid bone. 1 Reduced ossification of interparietal bone. *1 Reduced ossification of interparietal bone, reduced ossification of supraoccipital bone, reduced ossification of hyoid bone, reduced ossification of parietal bone.
4799	9	9 No visible skeletal abnormalities.

*Not commonly encountered.

PART I - SECTION E
THREE-GENERATION REPRODUCTION IN RATS

DIMP

LBI PROJECT NO. 10734-06

SUMMARY

Dietary incorporation of DIMP at levels of 300 and 3000 ppm produced no dose-related reproductive response in the rat for three successive generations with two matings per generation. The first mating of the third generation resulted in significant pup losses from Day 4 to Day 21 for the treatment groups. This decrease was not evidenced for the second mating. Food consumption and body weight data for the parent (F0), second generation (F1b) and third generation (F2b) rats, indicated statistical equivalence with the controls for all treatment levels except the high dose F2b female group. Litter and necropsy observations for the first breeding of the three generations were unremarkable and free of any dose-dependent relationship.

1. OBJECTIVE

The objective of this study was to evaluate the effects of dietary incorporation of DIMP in the rat on several indicators of the reproductive process for three successive generations with two breedings per generation.

2. MATERIAL

Refer to Part I - Section A.

3. EXPERIMENTAL DESIGN

A. First Generation (F0 Parents, F1a and F1b Offspring)

One hundred and twenty weanling rats [CRL:COBS CD (SD) BR] were obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan on March 30, 1977, at the Falls Church facility of the Department of Toxicology. Thirty male and sixty female rats were selected as the F0 parents and assigned by randomization into the following three groups.

<u>Group Number</u>	<u>Animal Numbers</u>		<u>Dose (ppm)</u>
	<u>Males</u>	<u>Females</u>	
1	5020-5029	5030-5049	0
2	5050-5059	5060-5079	300
3	5080-5089	5090-5109	3000

The remaining rats were sacrificed and discarded. The F0 parents were ear tagged and individually housed in hanging shoebox cages with AB-SORB-DRI bedding. The rats were maintained in a temperature-controlled animal room with artificial

3. EXPERIMENTAL DESIGN (Continued)

A. First Generation (F0 Parents, Fla and Flb Offspring - Continued)

illumination automatically controlled to provide a 12-hour light cycle. The F0 parents were fed Purina Laboratory Chow for one week and observed for any gross effects prior to initiation of the test diets. The test diet and acidified water (pH 2.5) were provided ad libitum. The time framework for this study has been presented in Text Table A.

Ten kg of the test diet for each dose level were prepared weekly by adding the requisite amount of DIMP for a given dose level to 300 ml of a PEG 400 vehicle and mixing the vehicle and basal diet in a twin-shell blender for 20 minutes. Control diet was prepared with the suspension vehicle in a similar fashion. Appendix B lists the concentration data as a function of the study week and also describes the analytical procedure for quantitating the DIMP level. Stability tests for the low and high level feed mixtures from the fifteenth study week were performed for samples stored in open and closed containers. The results (Table 1 of Appendix B) were indicative of considerable sample volatility from open containers. The mean sample concentrations and associated standard errors for the 300 and 3000 ppm feed mixtures were 251 ± 5 ppm and 2667 ± 36 ppm, respectively, for the time course of this study.

During the twelfth study week, the F0 parents were placed in breeding cages, one male and two females, for a two-week mating period. The males and females were then returned to their individual cages where the females were allowed to litter and nurse their pups. One-third of the Fla pups were subjected to a cursory necropsy after weaning. One week after lactation of the Fla pups, the F0 females were remated in a similar manner with a different male. During Day 4 of lactation the Fla and Flb litters were reduced for subsequent viability and weight measurements at Days 9 and 21. One week after lactation of the Flb pups all F0 parents were killed and necropsied.

The data and observations listed below were recorded for the parents and their offspring.

F0 Parents

Body weights and food consumption (averaged over seven days)
at study Weeks 4, 9, 11 and 20.

Daily observations for mortality.

Weekly appearance observations.

Anatomic observations at necropsy.

TABLE I-E-7

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE A

TIME FRAMEWORK FOR STUDY

<u>STUDY WEEK NO(S).</u>	<u>PHASE</u>
1	Fo parents begin DIMP dietary input
12, 13, 14	F1a mating period (Fo parents)
16, 17	Lactation Day 0 for F1a pups
19, 20	F1a pup necropsy
21, 22, 23	F1b mating period (Fo parents)
24, 25, 26	Lactation Day 0 for F1b pups
27, 28, 29	Fo parents necropsied
40, 41, 42	F2a mating period (F1b parents)
43, 44, 45	Lactation Day 0 for F2a pups
47, 48	F2a pup necropsy
49, 50, 51	F2b mating period (F1b parents)
53, 54	Lactation Day 0 for F2b pups
56, 57, 58	F1b parents necropsied
70, 71, 72	F3a mating period (F2b parents)
73, 74	Lactation Day 0 for F3a pups
77, 78	F3a pup necropsy
78, 79, 80	F3b mating period (F2b parents)
81, 82, 83	Lactaton Day 0 for F3b pups
85, 86	F3b pup necropsy
87	F2b parents necropsied

3. EXPERIMENTAL DESIGN (Continued)

A. First Generation (F0 Parents, F1a and F1b Offspring - Continued)

F1a and F1b Pups

Number of live and dead pups at birth, sex and body weights.

Number and sex of surviving pups at lactation Day 4.

Number, sex and body weights for reduced litter pups surviving at Day 21.

Daily mortality and appearance observations.

Anatomic observations at necropsy of one-third of F1a litters.

B. Second Generation (F1b Parents, F2a and F2b Offspring)

After weaning, 10 male and 20 female F1b pups were selected from each dose and control group, and identified by ear tag as listed below. The rats were individually housed for the second generation breedings.

<u>Group Number</u>	<u>Animal Numbers</u>		<u>Dose (ppm)</u>
	<u>Males</u>	<u>Females</u>	
1	5446-5455	5456-5475	0
2	5476-5485	5486-5505	300
3	5506-5515	5516-5535	3000

The same schedule for mating, littering and observations, as previously described for the first generation rats, was followed for the second generation rats.

C. Third Generation (F2b Parents, F3a and F3b Offspring)

After weaning, 10 male and 20 female F2b pups were selected from each dose and control group, and ear tagged as listed below. They were individually housed for the third generation breedings.

<u>Group Number</u>	<u>Animal Numbers^a</u>		<u>Dose (ppm)</u>
	<u>Males</u>	<u>Females</u>	
1	1407-1406	1407-1426	0
2	1427-1436	1437-1456	300
3	1457-1466	1467-1487	3000

^a"A" series animal number ear tags.

The same schedule for mating, littering and observations, as previously described for the first generation rats, was followed for the third generation rats. In addition, one-third of the F3b litters were killed and necropsied at weaning.

3. EXPERIMENTAL DESIGN (Continued)

C. Third Generation (F2b Parents, F3a and F3b Offspring - Continued)

The newborn viability ratios (live pups/total pups), pup viability ratios (pups at Day 4/pups at Day 0), lactation indices (pups at Day 21/pups at Day 4) and gestation indices (females littering/pregnant) for the treatment groups were statistically compared with the controls using 2x2 contingency tables with Chi-squared corrected for continuity. Dunnett's t-test for analysis for statistical differences was used to compare control and treatment means for the parent body weights, parent food consumption and pup weights. A p value of 0.05 was used to determine statistical significance.

4. RESULTS

A. First Generation (F0 Parents, F1a and F1b Offspring)

The detailed litter data, parent body weights, parent food consumption data, litter and parent observations, and necropsy data for parents and pups have been incorporated in Appendix A for this and succeeding generations. Litter summaries have been presented as text tables.

One female rat in the high dose group (No. 5109) died during the sixth week of the study. No visible abnormalities were noted and the death was judged not to be related to treatment. One male control rat (No. 5027) failed to successfully mate for two breedings. Necropsy data for this rat failed to show any evidence of sexual or reproductive dysfunction.

During the second week of study female control rat (No. 5030) developed ocular changes in the right eye which persisted until necropsy. A consultant ophthalmologist examined the animal and he indicated the presence of a unilateral cataract. His report has been appended.

The litter summaries for the first and second matings, Text Tables B and C, respectively, with detailed listing in appended Tables 1 and 4, showed a slight weight reduction of approximately 12% for the F1b control pups, both males and females at Day 21, versus the F1b pups. All other indices of reproductive performance presented in the summary tables exhibited statistical equivalence between control and treatment levels for both male and female groups. The pup viability counts for Days 4 and 21 were greater than 96% in all instances. Pup viability counts are usually calculated from the differential totals at Days 4 and 21 because of pup cannibalization by the dam. For this reason, the litter observation tables, appended Tables 2 and 5 do not correspond with the viability counts presented in the summaries.

The parent body weights and daily food consumption means, appended in Table 6, for the treatment groups were statistically equivalent to the controls for both male and female groups at all measurement levels.

TABLE I-E-8

LITTON BIONETICS
PROJECT NO. 10734-06

TABLE B

SUMMARY OF FIRST GENERATION - FIRST MATING (Fla)

	DOSE (PPM)					
	0		300		3000	
	RATIO	PERCENT	RATIO	PERCENT	RATIO	PERCENT
<u>Indices</u>						
Male virility (males producing litter/ mated)	9/10	90	10/10	100	10/10	100
Female fertility (females producing litter/mated)	17/20	85	17/20	85	16/19	84
Gestation (females live litter/pregnant)	17/17	100	17/17	100	16/16	100
Newborn viability (live pups/total pups)	227/227	100	231/237	97	206/208	99
Pup viability (pups Day 4/pups Day 0)	226/227	100	226/231	98	206/206	100
Lactation (pups Day 21/pups Day 4) ^a	133/134	99	134/135	99	128/128	100
<u>Pup weight in grams (Mean \pm S.D.)</u>						
Day 0 males	7 \pm 1.2		6 \pm 0.6		7 \pm 0.7	
Day 0 females	6 \pm 0.9		6 \pm 0.0		7 \pm 0.6	
Day 21 males	53 \pm 6.9		50 \pm 4.7		53 \pm 5.8	
Day 21 females	49 \pm 4.7		48 \pm 4.7		51 \pm 5.6	
Sex ratio offspring (M/F) Day 0	104/123		107/124		100/106	
Live pups per litter (Mean \pm S.D.)	13 \pm 2.6		14 \pm 2.7		13 \pm 1.8	

^aAfter litters were reduced at Day 4.

TABLE I-E-9

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE C

SUMMARY OF FIRST GENERATION - SECOND MATING (Flb)

Indices	DOSE (PPM)			
	0		300	
	RATIO	PERCENT	RATIO	PERCENT
Male virility (males producing litter/ mated)	9/10	90	10/10	100
Female fertility (females producing litter/mated)	16/18	89	18/20	90
Gestation (females live litter/pregnant)	16/16	100	18/18	100
Newborn viability (live pups/total pups)	204/206	99	243/246	99
Pup viability (pups Day 4/pups Day 0)	195/204	96	239/243	98
Lactation (pups Day 21/pups Day 4) ^a	118/120	98	144/144	100
Pup weight in grams (Mean \pm S.D.)				
Day 0 males	7 \pm 0.7		7 \pm 0.6	
Day 0 females	6 \pm 0.6		7 \pm 0.7	
Day 21 males	46 \pm 7.0		49 \pm 5.7	
Day 21 females	43 \pm 7.8		48 \pm 5.7	
Sex ratio offspring (M/F) Day 0	96/108		107/136	
Live pups per litter (Mean \pm S.D.)	14 \pm 1.8		14 \pm 2.2	
			108/101	
			12 \pm 3.0	

^aAfter litters were reduced at Day 4.

4. RESULTS (Continued)

A. First Generation (F0 Parents, F1a and F1b Offspring - Continued)

Parent observations have been appended in Table 7. A slight incidence of alopecia was observed throughout the study. The alopecia frequently disappeared with time.

Pup necropsy observations for one-third of the F1a control and treatment groups have been presented in Appendix Table 3. No gross abnormalities were recorded for the 17 litters that were examined.

Adult necropsy findings have been noted in Appendix Table 8. The major finding was the high incidence of kidney mottling. The number of occurrences was equally distributed across the control and treatment groups.

B. Second Generation (F1b Parent, F2a and F2b Offspring)

It was discovered that litter mates had inadvertently been paired for the F2a and F3b matings, appended Tables 9 and 20. Text Table D lists the brother-sister pairs and whether the matings were successful. The pairings were judged not to compromise the integrity of the study because the majority of the occurrences were in the F2a mating, whose litters were not subsequently bred, and because the majority of these matings (85%) successfully littered. It was interesting to note that 16 control pups from the litter-mate pairings died between Days 0 and 4. Inclusion of this number in the pup viabilities (Text Table E, Day 4/Day 0) raised this ratio to 91%. This percentage, however, was still significantly depressed, using the Chi-square statistic, from the treatment ratios. No F1b parents died on the study.

For the first mating, female parent No. 5527 (3000 ppm) produced stillborn Siamese twins which were joined at the ventral thoracic region. One twin was well developed anatomically, but the second twin lacked a discernible head. The remaining observations for the F2a and F2b litters, detailed on Appendix Tables 10 and 13, were not indicative of any compound-related effects and appeared unremarkable.

Within the 300 ppm group, male rat Nos. 5476 and 5477 failed to successfully mate for the first breeding. Male parent No. 5507 (3000 ppm) also failed to successfully mate. This was reflected in the newborn pup counts (Text Table E) which were decreased from the controls by 20%. The number of live pups per litter was equivalent.

TABLE I-E-10

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE D

LITTER MATE PAIRINGS

F2a MATING

0 PPM			
MALE NUMBER	FEMALE NUMBER	LITTER PRODUCED	
5447	5458	Yes	
5448	5460	Yes	
5449	5462	Yes	
5450	5464	Yes	
5451	5466	Yes	
5452	5468	No	
5453	5469	Yes	
5454	5470	Yes	
5455	5471	No	
5456	5472	Yes	
5457	5473	Yes	

F3b MATING

300 PPM			
MALE NUMBER	FEMALE NUMBER	LITTER PRODUCED	
5477	5488	No	
5478	5490	Yes	
5479	5492	Yes	
5480	5494	Yes	
5481	5496	Yes	
5482	5498	Yes	
5483	5500	Yes	
5484	5502	Yes	
5485	5503	Yes	
1430	1444	Yes	
1431	1445	Yes	

3000 PPM			
MALE NUMBER	FEMALE NUMBER	LITTER PRODUCED	
5507	5518	No	
5508	5520	Yes	
5509	5522	Yes	
5510	5524	Yes	
5511	5526	Yes	
5512	5528	Yes	
5513	5529	No	
5514	5530	Yes	
5515	5531	Yes	
5516	5532	Yes	
5517	5533	Yes	
1465	1485	Yes	

TABLE I-E-11

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE E

SUMMARY OF SECOND GENERATION - FIRST MATING (F2a)

	DOSE (PPM)			
	300		3000	
	RATIO	PERCENT	RATIO	PERCENT
<u>Indices</u>				
Male virility (males producing litter/ mated)	10/10	100		
Female fertility (females producing litter/mated)			8/10	80
Gestation (females live litter/pregnant)	18/20	90	15/16	94
Newborn viability (live pups/total pups)	18/18	100	15/15	100
Pup viability (pups Day 4/pups Day 0)	223/233	96	173/178	97
Lactation (pups Day 21/pups Day 4) ^a	186/223	83	167/173	97
	112/121	93	109/118	92
			9/10	90
			16/18	89
			16/16	100
			184/189	97
			175/184	95
			124/125	99
<u>Pup weight in grams (Mean \pm S.D.)</u>				
Day 0 males	6 \pm 0.9			
Day 0 females	6 \pm 1.1		6 \pm 1.2	7 \pm 0.7
Day 21 males	39 \pm 7.5		6 \pm 1.0	7 \pm 0.9
Day 21 females	38 \pm 7.7		43 \pm 8.1	39 \pm 4.1
			39 \pm 8.1	38 \pm 3.8
Sex ratio offspring (M/F) Day 0	106/117		82/91	79/105
Live pups per litter (Mean \pm S.D.)	12 \pm 2.6		12 \pm 2.4	12 \pm 2.0

^aAfter litters were reduced at Day 4.

4. RESULTS (Continued)

B. Second Generation (F1b Parents, F2a and F2b Offspring- Continued)

The F2b mating (Text Table F and Appendix Table 12) produced a greater number of pregnant dams which was reflected in the larger total pup counts for the 300 and 3000 ppm levels. Concurrently, there was a slight, but insignificant, increase in the average litter size. Average pup weights at Day 21 for the F2b males and females were 9 and 19% greater than those from the F2a mating. Pup viabilities for both matings ranged from 92 to 99%.

Body weight and food consumption data have been tabulated in Appendix Table 14. Analysis of the data showed that the only statistical difference from the controls occurred at Week 4 for the high dose male parents. This significance disappeared by Week 9.

Clinical signs have been appended in Table 15. Localized hair loss (female No. 5489) and ulceration of the right ear with subsequent loss of this ear (female No. 5524) were the only observable clinical signs recorded for the F1b parents.

Pup necropsy observations for the F2a litters have been appended in Table 11. No significant observations were noted.

There were several instances of ovarian, uterine and Fallopian tube cysts observed at necropsy for the F1b dams. These findings have been detailed in Appendix Table 16. These findings were not considered to be treatment related since the number of occurrences was distributed equally between treatment and control groups.

C. Third Generation (F2b Parents, F3a and F3b Offspring)

No F2b parents died during the study. The third generation matings resulted in newborn viabilities of 98 to 100% and equivalent average litter size for all groups. These findings have been summarized in Text Tables G and H and appended in Tables 17 and 20. Many pup losses were noted for the 3000 ppm F2b group from Day 0 to Day 4, significantly reducing this viability ratio from the control (Appendix Table 2). Lactation viabilities for the F2a treatment groups from Day 4 to Day 21 were significantly decreased from the controls. The Chi-square significance in the 300 ppm group could be attributed to the total litter loss of female No. 1443. Application of Wilcoxon Rank Sum analysis did not indicate a significant difference. The pup losses at the high treatment level were distributed among eight dams.

TABLE I-E-12

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE F

SUMMARY OF SECOND GENERATION - SECOND MATING (F2b)

	DOSE (PPM)			
	0	300	3000	
	RATIO	PERCENT	RATIO	PERCENT
<u>Indices</u>				
Male virility (males producing litter/ mated)	10/10	100	10/10	100
Female fertility (females producing litter/mated)	18/20	90	17/20	85
Gestation (females live litter/pregnant)	18/18	100	17/17	100
Newborn viability (live pups/total pups)	250/256	98	228/230	99
Pup viability (pups Day 4/pups Day 0)	242/250	97	222/228	97
Lactation (pups Day 21/pups Day 4)	142/144	99	135/136	99
Pup weight in grams (Mean \pm S.D.)				
Day 0 males	6 \pm 0.6		7 \pm 1.0	7 \pm 0.8
Day 0 females	6 \pm 0.6		7 \pm 1.0	6 \pm 0.6
Day 21 males	44 \pm 5.5		47 \pm 6.3	48 \pm 8.7
Day 21 females	42 \pm 5.4		44 \pm 5.0	45 \pm 7.0
Sex ratio offspring (M/F) Day 0	119/131		117/111	117/116
Live pups per litter (Mean \pm S.D.)	14 \pm 2.4		13 \pm 3.1	13 \pm 2.5

^aAfter litters were reduced at Day 4.

TABLE I-E-13

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE G

SUMMARY OF THIRD GENERATION - FIRST MATING (F3a)

	DOSE (PPM)					
	0		300		3000	
	<u>RATIO</u>	<u>PERCENT</u>	<u>RATIO</u>	<u>PERCENT</u>	<u>RATIO</u>	<u>PERCENT</u>
<u>Indices</u>						
Male virility (males producing litter/ mated)	10/10	100	10/10	100	9/10	90
Female fertility (females producing litter/mated)	18/20	90	17/20	85	18/20	90
Gestation (females live litter/pregnant)	18/18	100	17/17	100	18/18	100
Newborn viability (live pups/total pups)	210/212	99	212/212	100	219/221	99
Pup viability (pups Day 4/pups Day 0)	202/210	96	204/212	96	217/219	99
Lactation (pups Day 21/pups Day 4) ^a	128/129	99	116/129	90*	108/138	78*
<u>Pup weight in grams (Mean \pm S.D.)</u>						
Day 0 males	6 \pm 0.9		6 \pm 0.7		7 \pm 1.0	
Day 0 females	6 \pm 1.0		6 \pm 0.8		6 \pm 1.1	
Day 21 males	42 \pm 6.2		39 \pm 7.2		32 \pm 7.9	
Day 21 females	41 \pm 7.1		37 \pm 7.2		30 \pm 8.1	
Sex ratio offspring (M/F) Day 0	108/102		127/85		124/95	
Live pups per litter (Mean \pm S.D.)	12 \pm 3.7		13 \pm 4.7		12 \pm 3.3	

^aAfter litters were reduced at Day 4.

*p<0.05; Chi-square with correction for continuity.

TABLE I-E-14

SUMMARY OF THIRD GENERATION - SECOND MATING (F3b)

^aAfter litters were reduced at Day 4.

*p<.0.05, Chi-square with correction for continuity.

4. RESULTS (Continued)

C. Third Generation (F2b Parents, F3a and F3b Offspring)

The F3b pup weights at Day 21 were 9 to 30% greater than the F3a pup weights. A similar trend was observed in the second generation matings.

The only statistically significant feature of the parent body weight and food consumption data, appended in Table 23, was the 7% weight reduction of the high dose level females recorded at Week 20. Dunnett's t-test of analysis of the Week 20 male and female parent weights showed that there was a statistical equivalence among the three generations for the control and treatment groups.

Pup appearance and necropsy observations did not show any evidence of treatment-related effects for the F3a and F3b litters. These data have been detailed in Tables 18, 19, 21 and 22, respectively, of the Appendix.

Observations for the F2b parents have been detailed in Appendix Table 24. The clinical signs listed were not judged to be treatment related.

There were numerous incidences of kidney mottling recorded during the necropsy of the F2b parents (Appendix Table 25). They were divided across treatment and control groups for both sexes, and were therefore judged unrelated to treatment. The only other significant finding was the presence of a mammary mass in female No. 1444 (3000 ppm).

Thirty days after transmittal of this report, original data from the Department of Toxicology will be transferred to the LBI Archivist, 5516 Nicholson Lane, Kensington, Maryland, for distribution to the proper repositories. A copy of this report was reviewed by the LBI Quality Assurance Unit.

5. CONCLUSION

Dietary incorporation of DIMP at levels of 300 and 3000 ppm produced no dose-related reproductive response in the rat for three successive generations with two matings per generation. The first mating of the third generation resulted in significant pup losses from Day 4 to Day 21 for the treatment groups. This decrease was not evidenced for the second mating. Food consumption and body weight data for the parent (F0), second generation (F1b) and third generation (F2b) rats, indicated statistical equivalence with the controls for all treatment levels except the high dose F2b female group. Litter and necropsy observations for the first breeding of the three generations were unremarkable and free of any dose-dependent relationship.

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APPENDIX A

TABLE I-E-15

LITTON BIONEUTICS, INC.
PROJECT NO. 10734-06

TABLE 1

RESULTS OF FIRST GENERATION - FIRST MATING (F1a)

DOSE - 0 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21			
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F
5030	5020	16		8/8	6	6		16	8/8	4/4		8	4/4
5031	5020	15		7/8	6	6		15	7/8	4/4		8	4/4
5032	5021	11		2/9	6	6		11	2/9	2/6		8	4/4
5033	5021	14		9/5	6	6	1	13	8/5	4/4		8	4/4
5034	5022	6		2/4	11	9		6	2/4	2/4		6	2/4
5035	5022												
5036	5023	14		9/5	6	5		14	9/5	4/4		8	4/4
5037	5023	16		10/6	6	5		16	10/6	4/4		8	4/4
5038	5024	14		9/5	6	6		14	9/5	4/4	1	7	4/3
5039	5024	14		8/6	7	6		14	8/6	4/4		8	4/4
5040	5025	16		9/7	6	6		16	9/7	4/4		8	4/4
5041	5025	10		2/8	6	6		10	2/8	2/6		8	4/4
5042	5026	12		3/9	7	6		12	3/9	3/5		8	2/6
5043	5026	15		6/9	6	6		15	6/9	4/4		8	3/5
5044	5027												
5045	5027												
5046	5028	14		6/8	7	6		14	6/8	4/4		8	4/4
5047	5028	14		5/9	7	6		14	5/9	4/4		8	4/4
5048	5029	14		4/10	7	7		14	4/10	4/4		8	4/4
5049	5029	12		5/7	7	6		12	5/7	4/4		8	4/4
TOTAL		227		104/123	7	6	1	226	103/123	61/73	1	133	61/72
MEAN		13			7	6		13				8	
SD		2.6			1.2	0.9		2.5				0.5	
SE		0.6			0.3	0.2		0.6				0.1	
N		17						17				17	

TABLE I-E-15 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-06

TABLE 1 (Continued)

RESULTS OF FIRST GENERATION - FIRST MATING (Fla)

DOSE - 300 PPH

FFEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5060	5050	16		8/8	6	6		16	8/8	4/4		8	4/4	49	45
5061	5050	12		6/6	6	6		12	6/6	4/4		8	4/4	52	50
5062	5051	13	1	7/6	6	6		13	7/6	4/4		8	4/4	56	51
5063	5051	14		7/7	7	6		14	7/7	4/4		8	4/4	49	45
5064	5052	14		7/7	6	6		14	7/7	4/4		8	4/4	46	47
5065	5052	14	1	7/7	6	6		14	7/7	4/4		8	4/4	45	44
5066	5053	14	1	5/6	6	6	1	10	4/6	4/4		8	4/4	48	53
5067	5053	11	4	5/2	6	6	1	7	5/2	5/2	1	6	4/2	47	60
5068	5054	7		7/7	6	6	1	13	7/6	4/4		8	4/4	58	44
5069	5054	14		5/10	6	6	1	15	5/10	4/4		8	4/4	49	44
5070	5055	15		7/10 a	5	6	1	16	7/9	4/4		8	4/4	44	44
5071	5055	17		4/9	7	6	1	12	3/9	3/5		8	3/5	50	46
5072	5056	13													
5073	5056														
5074	5057	16		6/10	7	6		16	6/10	4/4		8	4/4	47	45
5075	5057	18		7/11	6	6	1	17	6/11	4/4		8	4/4	48	47
5076	5058	15		5/10	7	6		15	5/10	4/4		8	4/4	60	57
5077	5058														
5078	5059	11		6/5	7	6		11	6/5	4/4		8	4/4	56	51
5079	5059	11		8/3	6	6		11	8/3	5/3		8	5/3	52	49
TOTAL		231	6	107/124			5	226	104/122	69/66	1	134	68/66		
MEAN		14			6	6		13				8		50	48
SD		2.7			0.6	0.0		2.6				0.5		4.7	4.7
SE		0.7			0.1	0.0		0.6				0.1		1.1	1.1
N		17						17				17			

^apup mis-sexed at Day 0.

TABLE I-E-15 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-06

TABLE 1 (Continued)

RESULTS OF FIRST GENERATION - FIRST MATING (F1a)

DOSE - 3000 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5090	5080	13		4/9	6	6		13	4/9	4/4		8	4/4	52	52
5091	5080	14		10/4	7	7		14	10/4	4/4		8	4/4	51	48
5092	5081	11		6/5	8	8		11	6/5	4/4		8	4/4	63	60
5093	5082	13		9/4	8	7		13	9/4	4/4		8	4/4	57	59
5094	5083	18		11/7	6	6		18	11/7	4/4		8	4/4	54	51
5095	5083	14		5/9	7	6		14	5/9	4/4		8	4/4	59	57
5096	5083	11		5/6	7	6		11	5/6	4/4		8	4/4	59	50
5097	5084	12		5/7	7	6		12	5/7	4/4		8	4/4	49	45
5098	5084	14		4/10	8	7		14	4/10	4/4		8	4/4	46	45
5099	5085	12		4/8	7	7		12	4/8	4/4		8	4/4	51	52
5100	5085	13		8/5	7	6		13	8/5	4/4		8	4/4	51	47
5101	5086	13		7/6	8	7		13	7/6	4/4		8	4/4	51	48
5102	5087	12	2	3/9	7	6		12	3/9	3/5		8	3/5	59	56
5103	5087	14		7/7	7	6		14	7/7	4/4		8	4/4	48	46
5104	5088	11		7/4	7	7		11	7/4	4/4		8	4/4	58	56
5105	5088	11		5/6	6	6		11	5/6	4/4		8	4/4	41	41
5106	5089														
5106a															
5109a															
TOTAL		206	2	100/106	7	7		206	100/106	63/65		128	63/65		
MEAN		13						13				8		53	51
SD		1.8			0.7	0.6		1.8				0.0		5.8	5.6
SE		1.5			0.2	0.2		0.5				0.0		1.4	1.4
N		16						16				16			

^aDeath recorded during sixth study week.

TABLE I-E-16

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 2

LITTER OBSERVATIONS

FIRST GENERATION - FIRST MATING (Fla)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATION</u>
0	5033	2	ONE MALE PUP - FOUND DEAD; NO ABNORMALITIES NOTED.
	5038	19	ONE FEMALE PUP - FOUND DEAD.
	5042	21	ONE FEMALE PUP - OPACITY RIGHT EYE.
300	5063	0	ONE MALE PUP - FOUND DEAD.
	5066	0	ONE MALE PUP - FOUND DEAD.
	5068	0	ONE MALE PUP - FOUND DEAD.
		0	ONE FEMALE PUP - FOUND DEAD.
	5071	2	ONE MALE PUP - FOUND DEAD.
	5072	2	ONE MALE PUP - FOUND DEAD.
	5075	2	ONE MALE PUP - FOUND DEAD.
3000	5105	0	TWO MALE PUPS - FOUND DEAD.

TABLE I-E-17

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 3

PUP NECROPSY OBSERVATIONS (Fla) AT DAY 21

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>NUMBER OF PUPS</u>	<u>OBSERVATION</u>
0	5030	8	ALL TISSUES APPEAR NORMAL
	5031	8	ALL TISSUES APPEAR NORMAL
	5033	8	ALL TISSUES APPEAR NORMAL
	5037	8	ALL TISSUES APPEAR NORMAL
	5039	8	ALL TISSUES APPEAR NORMAL
300	5060	8	ALL TISSUES APPEAR NORMAL
	5064	8	ALL TISSUES APPEAR NORMAL
	5069	8	ALL TISSUES APPEAR NORMAL
	5070	8	ALL TISSUES APPEAR NORMAL
	5074	8	ALL TISSUES APPEAR NORMAL
	5075	8	ALL TISSUES APPEAR NORMAL
3000	5093	8	ALL TISSUES APPEAR NORMAL
	5095	8	ALL TISSUES APPEAR NORMAL
	5098	8	ALL TISSUES APPEAR NORMAL
	5104	8	ALL TISSUES APPEAR NORMAL
	5106	8	ALL TISSUES APPEAR NORMAL
	5107	8	ALL TISSUES APPEAR NORMAL

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE I-E-18

TABLE 4

RESULTS OF FIRST GENERATION - SECOND MATING (F1b)

DOSE - 0 ppm

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5030	5029	15		7/8	7	6		15	7/8	4/4		8	4/4	50	45
5031	5029	16		7/9	6	6		16	7/9	4/4		8	4/4	51	45
5032	5028	12		5/7	6	7	2	10	4/6	4/4		8	4/4	45	40
5033	5028	15		6/9	6	6		15	6/9	4/4	1	7	3/4	46	48
5034	5027														
5035	5027														
5036	5026	15		9/6	6	6	1	14	8/6	4/4		8	4/4	50	48
5037	5026	14		4/10	6	6	1	13	3/10	3/5		8	4/4 ^a	40	37
5038	5025	13		9/4	6	6		13	9/4	4/4		8	4/4	40	40
5039	5025														
5040	5024	16		11/5	8	7		16	11/5	4/4		8	4/4	51	47
5041	5024	13		7/6	6	6	1	12	6/6	4/4		8	4/4	42	38
5042	5023	15		4/11	6	6	1	14	4/10	4/4		8	4/4	50	45
5043	5023	11		5/6	7	6		11	5/6	4/4		8	4/4	46	43
5044	5022	11		4/7	8	8		11	4/7	4/4		8	4/4	45	43
5045	5022														
5046	5021	10		4/6	7	8	1	9	3/6	3/5		7	3/4	56	54
5047	5021	13		7/6	6	6	2	11	6/5	4/4		8	4/4	29	26
5048	5020	12	2	6/6	7	6		12	6/6	4/4		8	4/4	39	32
5049	5020	14		5/9	8	7		14	5/9	4/4		8	4/4	53	52
TOTAL		204	2	96/108			9	195	90/105	58/62	2	118	58/60		
MEAN		14			7	6		13				8		46	43
SD		1.8			0.7	0.6		2.1				0.4		7.0	7.8
SE		0.5			0.2	0.2		0.6				0.1		1.9	2.0
N		16						16				16			

^aPup mis-sexed at Day 4; corrected at Day 21.

TABLE I-E-18 (Continued)

LITTON BIOHETICS, INC.
PROJECT NO. 10734-06

TABLE 4 (Continued)

RESULTS OF FIRST GENERATION - SECOND MATING (F1b)

DOSE - 300 PPH

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5060	5059	12	2	7/5	7	7		12	7/5	4/4		8	4/4	46	45
5061	5059	13		8/5	7	6		13	8/5	4/4		8	4/4	52	49
5062	5058														
5063	5058	17		4/13	7	6	1	16	4/12	4/4		8	4/4	54	48
5064	5057	19		8/11	6	6	1	18	7/11	4/4		8	4/4	35	33
5065	5057	15		0/15 ^a		8		15	0/15	4/4		8	4/4	51	51
5066	5056	10		3/7	6	7		10	3/7	3/5		8	3/5	49	50
5067	5056	12		4/8	6	6		12	4/8	4/4		8	4/4	49	45
5068	5055	15		9/6	6	6	1	14	8/6	4/4		8	4/4	50	50
5069	5055	15		6/9	7	6		15	6/9	4/4		8	4/4	52	50
5070	5054	14		7/7	6	6	1	13	7/6	4/4		8	4/4	50	46
5071	5054														
5072	5053	12		8/4	6	6		12	8/4	4/4		8	4/4	40	40
5073	5053	13		6/7	6	6		13	6/7	4/4		8	4/4	53	54
5074	5052	13		5/8	7	8		13	5/8	4/4		8	4/4	42	42
5075	5052	10		6/4	7	7		10	6/4	4/4		8	4/4	54	54
5076	5051	13	1	6/7	8	7		13	6/7	4/4		8	4/4	51	52
5077	5051	14		7/7	7	7		14	7/7	4/4		8	4/4	53	52
5078	5050	12		6/6	7	7		12	6/6	4/4		8	4/4	56	54
5079	5050	14		7/7	6	6		14	7/7	4/4		8	4/4	41	41
TOTAL		243	3	107/136			4	239	105/134	71/73	0	144	71/73		
MEAN		14			7	7		13				8		49	48
SD		2.2			0.6	0.7		2.0				0.0		5.7	5.7
SE		0.5			0.2	0.2		0.5				0.0		1.3	1.3
N		18						18				18			

^aPups mis-sexed at Day 0.

TABLE I-E-18 (Continued)

LITTON BIOHETICS, INC.
PROJECT NO. 10734-06

TABLE 4 (Continued)

RESULTS OF FIRST GENERATION - SECOND MATING (F1b)

DOSE - 3000 PPH

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER F/M	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5090	5089	11		4/7	7	6		11	4/7	4/4		8	4/4	57	52
5091	5089	14		6/8	8	8		14	6/8	4/4		8	4/4	41	45
5092	5088	14		7/7	7	7		14	7/7	4/4		8	4/4	53	51
5093	5088	16		11/5	7	7		16	11/5	4/4		8	4/4	54	50
5094	5087	12		4/8	7	7		12	4/8	4/4		8	4/4	52	47
5097	5087	12		5/7	7	7		12	5/7	4/4		8	4/4	65	58
5096	5086	14		6/8	7	7		14	6/8	4/4		8	4/4	61	50
5097	5086														
5098	5085	11	2	7/4	7	6		11	7/4	4/4		8	4/4	54	49
5099	5085	16		11/5	7	6		16	11/5	4/4		8	4/4	34	29
5100	5084	14		8/6	7	7		14	8/6	4/4		8	4/4	54	51
5101	5084	13		7/6	7	6		13	7/6	4/4		8	4/4	61	59
5102	5083														
5103	5083	15		11/4	7	7	1	14	10/4	4/4		8	4/4	39	38
5104	5082	14		6/8	7	7		14	6/8	4/4		7	3/4	54	51
5105	5082	8		3/5	8	7		8	3/5	3/5		8	3/5	54	49
5106	5081	10	5	4/6	8	7		10	4/6	4/4	1	7	3/4	54	49
5107	5081	4		2/2	8	8		4	2/2	2/2		4	2/2	60	60
5108	5080	11		6/5	6	6		11	6/5	4/4		8	4/4	43	43
TOTAL		209	7	108/101			1	208	107/101	65/67	2	130	63/67		
MEAN		12			7	7		12				8		53	50
SD		3.0			0.6	0.7		3.2				1.0		8.2	7.8
SE		0.7			0.1	0.2		0.8				0.2		2.1	2.0
N		17						17				17			

TABLE I-E-19

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 5

LITTER OBSERVATIONS

FIRST GENERATION - SECOND MATING (F1b)

<u>DOSE</u> <u>(PPM)</u>	<u>FEMALE</u> <u>NUMBER</u>	<u>DAY OF</u> <u>LACTATION</u>	<u>OBSERVATION</u>
0	5040	0	ONE FEMALE PUP - SUBCUTANEOUS HEMATOMA, DORSAL-THORACIC AREA.
3000	5092	0	ONE FEMALE PUP - SUBCUTANEOUS HEMATOMA, TOP OF NOSE.
	5095	0	ONE FEMALE PUP - BLEEDING FROM LACERATION, RIGHT EAR.
	5096	0	ONE FEMALE PUP - SUBCUTANEOUS HEMATOMA, TOP OF NOSE.
	5096	0	ONE FEMALE PUP - SUBCUTANEOUS HEMATOMA, RIGHT EAR

TABLE I-E-20

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 6

BODY WEIGHTS AND FOOD CONSUMPTION (G) OF PARENT RATS (Fo)

BODY WEIGHTS

DOSE (PPM)		MALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	301	435	458	551
	SD	21.2	34.2	42.3	45.5
	SE	6.7	10.8	13.4	14.4
	N	10	10	10	10
300	MEAN	290	438	468	552
	SD	26.1	38.5	42.2	50.6
	SE	8.3	12.2	13.4	16.0
	N	10	10	10	10
3000	MEAN	288	426	453	532
	SD	20.0	30.8	38.7	42.4
	SE	6.3	9.7	12.2	13.4
	N	10	10	10	10

FOOD CONSUMPTION

DOSE (PPM)		MALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	22.0	25.4	23.8	23.2
	SD	2.2	2.5	3.8	4.0
	SE	0.7	0.8	1.2	1.3
	N	10	9	10	10
300	MEAN	21.7	24.9	24.2	22.9
	SD	1.5	1.0	1.4	2.4
	SE	0.6	0.4	0.5	0.8
	N	7	7	8	10
3000	MEAN	21.7	24.6	23.7	24.5
	SD	1.5	1.6	2.1	2.9
	SE	0.5	0.6	0.8	0.9
	N	10	8	7	10

TABLE I-E-20 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 6 (Continued)

BODY WEIGHTS AND FOOD CONSUMPTION (G) OF PARENT RATS (Fo)

BODY WEIGHTS

DOSE (PPM)		FEMALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	189	246	255	299
	SD	11.7	14.8	26.0	14.7
	SE	2.6	3.3	5.8	3.3
	N	20	20	20	20
300	MEAN	193	249	261	301
	SD	11.5	15.7	17.4	18.0
	SE	2.6	3.5	3.9	4.0
	N	20	20	20	20
3000	MEAN	195	252	263	296
	SD	19.0	26.0	26.1	24.3
	SE	4.2	6.0	6.0	5.6
	N	20	19	19	19

FOOD CONSUMPTION

DOSE (PPM)		FEMALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	16.1	19.3	19.3	21.2
	SD	2.0	2.5	3.5	3.2
	SE	0.5	0.6	0.8	0.7
	N	18	19	19	19
300	MEAN	15.9	19.1	18.8	20.6
	SD	1.8	3.1	3.0	3.2
	SE	0.4	0.7	0.7	0.7
	N	19	20	20	19
3000	MEAN	16.9	19.9	18.9	21.9
	SD	2.4	3.4	3.6	4.5
	SE	0.5	0.8	0.8	1.0
	N	20	19	19	19

TABLE 7

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NUMBER - 10734-06

WEEK NO. ** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
001 CONTROL	MALE 05020 *****	STARTED APRIL 8, 1977 3 GENERATION REPRODUCTION-RAT
001 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
002 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
300 PPM	MALE 05050	SKIN REDNESS : EYE-RIGHT
003 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
300 PPM	MALE 05052	SOFT STOOL
	FEMALE 05075	EAR TAG LOST
3000 PPM	05094	EAR TAG LOST
004 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
300 PPM	MALE 05055	ULCERATION : LATERAL-RIGHT, NECK
	FEMALE 05070	EAR TAG LOST
3000 PPM	MALE 05087	EAR TAG LOST
	FEMALE 05090	EAR TAG LOST
005 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
300 PPM	MALE 05055	ULCERATION : LATERAL-RIGHT, NECK
3000 PPM	FEMALE 05109	FOUND DEAD
006 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
300 PPM	MALE 05055	ULCERATION : LATERAL-RIGHT, NECK
3000 PPM	FEMALE 05093	LOCAL HAIR LOSS : LIMBS-FORE
007 CONTROL	MALE 05023	MISSING EAR TAG
	FEMALE 05030	EYE OPACITY-RIGHT EYE
300 PPM	MALE 05055	LOCAL HAIR LOSS : LATERAL-RIGHT, NECK
3000 PPM	FEMALE 05093	LOCAL HAIR LOSS : LIMBS-FORE
008 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
3000 PPM	05093	LOCAL HAIR LOSS : LIMBS-FORE
009 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
3000 PPM	05093	LOCAL HAIR LOSS : LIMBS-FORE
010 CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
3000 PPM	05091	EYE DISCHARGE : EYE-RIGHT: AROUND
	05093	LOCAL HAIR LOSS : LIMBS-FORE

TABLE I-E-21 (Continued)

TABLE 7 (CONTINUED)

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NO. - 10734-06

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
011	CONTROL	MALE 05020	MATE TO GET F1A
		FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
012	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
013	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
014	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
015	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
	3000 PPM	MALE 05083	EYE PARTIALLY CLOSED : EYE-LEFT
016	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
	3000 PPM	MALE 05083	EYE PARTIALLY CLOSED : EYE-LEFT
017	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	05066	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
	3000 PPM	MALE 05083	EYE PARTIALLY CLOSED : EYE-LEFT
018	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	300 PPM	MALE 05052	CRUST : NOSE:AROUND
		*****	RED
	3000 PPM	05083	EYE PARTIALLY CLOSED : EYE-LEFT
019	CONTROL	FEMALE 05030	LOCAL HAIR LOSS : LIMBS-FORE
			EYE OPACITY-RIGHT EYE
		05034	LOCAL HAIR LOSS : LIMB-FORE,LEFT
020	CONTROL	MALE 05020	MATE TO GET F1B
			MATE TO GET F1B
		FEMALE 05030	LOCAL HAIR LOSS : LIMBS-FORE
			EYE OPACITY-RIGHT EYE
		05034	LOCAL HAIR LOSS : LIMB-FORE,LEFT
021	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	3000 PPM	05102	LOCAL HAIR LOSS : LIMBS-FORE
		05103	LOCAL HAIR LOSS : LIMB-FORE,RIGHT
022	CONTROL	FEMALE 05030	EYE OPACITY-RIGHT EYE
	3000 PPM	05102	LOCAL HAIR LOSS : LIMBS-FORE
		05103	LOCAL HAIR LOSS : LIMB-FORE,RIGHT

TABLE I-E-21 (Continued)

TABLE 7 (CONTINUED)

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NO. - 10734-06

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
023	CONTROL 3000 PPM	FEMALE 05030 05102 05103	EYE OPACITY-RIGHT EYE LOCAL HAIR LOSS : LIMBS-FORE LOCAL HAIR LOSS : LIMB-FORE, RIGHT
024	CONTROL 3000 PPM	FEMALE 05030 05102 05103	EYE OPACITY-RIGHT EYE LOCAL HAIR LOSS : LIMBS-FORE LOCAL HAIR LOSS : LIMB-FORE, RIGHT
025	CONTROL 3000 PPM	FEMALE 05030 05102 05103	EYE OPACITY-RIGHT EYE LOCAL HAIR LOSS : LIMBS-FORE LOCAL HAIR LOSS : LIMB-FORE, RIGHT
026	CONTROL 3000 PPM	FEMALE 05030 05102 05103	EYE OPACITY-RIGHT EYE LOCAL HAIR LOSS : LIMBS-FORE LOCAL HAIR LOSS : LIMB-FORE, RIGHT
027	CONTROL 3000 PPM	FEMALE 05030 05102 05103	EYE OPACITY-RIGHT EYE LOCAL HAIR LOSS : LIMBS-FORE LOCAL HAIR LOSS : LIMB-FORE, RIGHT

TABLE I-E-22

LITTON BICNETICS, INC.
PROJECT NO. 10734-06

TABLE 8

PARENT NECROPSY OBSERVATIONS (F0) - MALES

<u>DOSE (PPM)</u>	<u>MALE NUMBER</u>	<u>OBSERVATION</u>
0	5020	ALL TISSUES APPEAR NORMAL
	5021	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5022	ALL TISSUES APPEAR NORMAL
	5023	ALL TISSUES APPEAR NORMAL
	5024	ALL TISSUES APPEAR NORMAL
	5025	ALL TISSUES APPEAR NORMAL
	5026	ALL TISSUES APPEAR NORMAL
	5027	ALL TISSUES APPEAR NORMAL
	5028	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
300	5029	ULCERATION (3 X 3 CM) RIGHT LATERAL THORACIC REGION; ALL TISSUES APPEAR NORMAL
	5050	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5051	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5052	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5053	ALL TISSUES APPEAR NORMAL
	5054	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5055	ALL TISSUES APPEAR NORMAL
	5056	ALL TISSUES APPEAR NORMAL
	5057	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
3000	5058	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5059	ALL TISSUES APPEAR NORMAL
	5080	ALL TISSUES APPEAR NORMAL
	5081	ALL TISSUES APPEAR NORMAL
	5082	ALL TISSUES APPEAR NORMAL
	5083	ALL TISSUES APPEAR NORMAL
	5084	ALL TISSUES APPEAR NORMAL
	5085	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5086	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5087	BOTH KIDNEYS PALE AND PITTED; ALL OTHER TISSUES APPEAR NORMAL
	5088	ALL TISSUES APPEAR NORMAL
	5089	ALL TISSUES APPEAR NORMAL

TABLE I-E-22 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 8 (Continued)

PARENT NECROPSY OBSERVATIONS (FO) - FEMALES

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>OBSERVATION</u>
0	5030	RIGHT EYE OPAQUE; ALL TISSUES APPEAR NORMAL
	5031	ALL TISSUES APPEAR NORMAL
	5032	ALL TISSUES APPEAR NORMAL
	5033	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5034	DIARRHEA, BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5035	ALL TISSUES APPEAR NORMAL
	5036	ALL TISSUES APPEAR NORMAL
	5037	ALL TISSUES APPEAR NORMAL
	5038	ALL TISSUES APPEAR NORMAL
	5039	ALL TISSUES APPEAR NORMAL
	5040	ALL TISSUES APPEAR NORMAL
	5041	ALL TISSUES APPEAR NORMAL
	5042	ALL TISSUES APPEAR NORMAL
	5043	ALL TISSUES APPEAR NORMAL
	5044	ALL TISSUES APPEAR NORMAL
	5045	ALL TISSUES APPEAR NORMAL
	5046	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5047	ALL TISSUES APPEAR NORMAL
	5048	ALL TISSUES APPEAR NORMAL
	5049	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
300	5060	ALL TISSUES APPEAR NORMAL
	5061	ALL TISSUES APPEAR NORMAL
	5062	ALL TISSUES APPEAR NORMAL
	5063	ALL TISSUES APPEAR NORMAL
	5064	NECROPSY NOT PERFORMED
	5065	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5066	ALL TISSUES APPEAR NORMAL
	5067	ALL TISSUES APPEAR NORMAL
	5068	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5069	ALL TISSUES APPEAR NORMAL

TABLE I-E-22 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 8 (Continued)

PARENT NECROPSY OBSERVATIONS (FO) - FEMALES

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>OBSERVATION</u>
300	5070	ALL TISSUES APPEAR NORMAL
	5071	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5072	ALL TISSUES APPEAR NORMAL
	5073	ALL TISSUES APPEAR NORMAL
	5074	ALL TISSUES APPEAR NORMAL
	5075	ALL TISSUES APPEAR NORMAL
	5076	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5077	ALL TISSUES APPEAR NORMAL
	5078	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5079	ALL TISSUES APPEAR NORMAL
3000	5090	ALL TISSUES APPEAR NORMAL
	5091	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5092	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5093	ALL TISSUES APPEAR NORMAL
	5094	ALL TISSUES APPEAR NORMAL
	5095	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5096	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5097	ALL TISSUES APPEAR NORMAL
	5098	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5099	ALL TISSUES APPEAR NORMAL
	5100	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5101	ALL TISSUES APPEAR NORMAL
	5102	ALL TISSUES APPEAR NORMAL
	5103	ALL TISSUES APPEAR NORMAL
	5104	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5105	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5106	ALL TISSUES APPEAR NORMAL
	5107	ALL TISSUES APPEAR NORMAL
	5108	ALL TISSUES APPEAR NORMAL

TABLE I-E-23

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 9

RESULTS OF SECOND GENERATION - FIRST MATING (F2a)

DOSE - 0 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5456	5455	11		4/7	6	5	2	9	3/6	3/5	8	0			
5457	5455	13		8/5	5	6		13	8/5	4/4 ^a		8	3/5	40	38
5458	5447	14		8/6	6	5	1	13	8/5	4/4		8	4/4	37	39
5459	5447	15		9/6	5	4		15	7/8	4/4		8	4/4	37	33
5460	5448	11		6/5	5	5	11	0				0			
5461	5448	12		8/4	6	5	4	8	6/2	6/2		8	6/2	45	41
5462	5449	12		6/6	6	5		12	6/6	4/4		8	4/4	40	38
5463	5449	13		6/7	6	6		13	6/7	4/4		8	4/4	47	43
5464	5450	13		6/7	6	6	1	12	5/7	4/4		8	4/4	39	36
5465	5450	13		4/9	8	8		13	4/9	4/4		8	4/4	25	26
5466	5451	14		8/6	7	7		14	8/6	4/4		8	4/4	41	39
5467	5451	3	7	2/1	5	5	3	0				0			
5468	5452														
5469	5452	13	2	6/7	6	6	1	12	5/7	4/4		8	4/4	43	39
5470	5453	12		6/6	7	7	2	10	6/4	4/4		8	4/4	42	42
5471	5453														
5472	5454	14		4/10	7	6		14	4/10	4/4		8	4/4	45	43
5473	5454	12		7/5	7	7		12	7/5	4/4		8	4/4	47	52
5474	5446	13		6/7	5	4	12	1	0/1	0/1	1	0			
5475	5446	15	1	2/13	7	6		15	2/13	2/6		8	2/6	22	20
TOTAL		223	10	106/117			37	186	85/101	59/62	9	112	55/57		
MEAN		12			6	6		10				6		39	38
SD		2.6			0.9	1.1		5.0				3.4		7.5	7.7
SE		0.6			0.2	0.2		1.2				0.8		2.0	2.1
N		18						18				18			

^aOne pup mis-sexed, corrected at Day 21.

TABLE I-E-23 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 9 (Continued)

RESULTS OF SECOND GENERATION - FIRST MATING (F2a)

DOSE - 300 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5486	5485	14		7/7	6	5		14	7/7	4/4		8	4/4	48	43
5487	5485	9		4/5	7	7		9	4/5	4/4		8	4/4	52	48
5488	5477														
5489	5477														
5490	5478	14	1	10/4 ^a	6	6		14	4/18	4/4		8	4/4	23	24
5491	5478	10		2/8	7	7		11	2/9	2/6		8	2/6	48	46
5492	5479	7		3/4 ^a	7	7		7	4/3	4/3		7	4/3	43	41
5493	5479	8		4/4	10	8		8	4/4	4/4		8	4/4	55	48
5494	5480	11	1	6/5	6	5		11	6/5	4/4		8	4/4	42	36
5495	5480	13		6/7	5	5	1	12	6/6	4/4		7	4/3	35	38
5496	5481	13		4/9	6	5		13	4/9	4/4		8	4/4	38	35
5497	5481	13	1	8/5	7	7		13	8/5	4/4		8	4/4	50	47
5498	5482	11		7/4	6	5		11	8/3	5/3		8	5/3	42	41
5499	5482	12	1	6/6	6	6		12	6/6	4/4	8	0			
5483	5500	15		4/11 ^a	5	5		15	3/12	3/5		8	3/5	41	37
5501	5483														
5502	5484	10		4/6	6	6	3	7	3/4	3/4		7	3/4	39	22
5503	5484	13	1	7/6	5	5	3	10	5/5	4/4		8	4/4	40	38
5504	5476														
5505	5476														
TOTAL		173	5	82/91			7	167	74/93	57/61	9	109	53/56		
MEAN		12			6	6		11				7		43	39
SD		2.4			1.2	1.0		2.5				2.1		8.1	8.1
SE		0.6			0.3	0.3		0.7				0.5		2.1	2.1
N		15						15				15			

^aMiss-sexed at Day 0, corrected at Day 4.
^blate birth recorded after Day 0.

TABLE I-E-23 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 9 (Continued)

RESULTS OF SECOND GENERATION - FIRST MATING (F2a)

DOSE - 3000 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5516	5515	13		5/8	7	7		13	5/8	4/4 ^a		8	5/3	46	45
5517	5515														
5518	5507														
5519	5507														
5520	5508	12		6/6 ^b	7	7		12	5/7	4/4		8	4/4	42	39
5521	5508	12		6/6	7	6		12	6/6	4/4		8	4/4	50	48
5522	5509	10		2/8 ^b	7	7		10	2/8	2/6		8	2/6	41	35
5523	5509	7	1	2/5	7	6		7	3/4	3/4 ^a		7	2/5	28	29
5524	5510	14	1	5/9	7	7	1	13	5/8	4/4		8	4/4	36	37
5525	5510	15		8/7	6	6		14	8/6	4/4		8	4/4	40	37
5526	5511	10		6/4	7	7	1	10	6/4	4/4		7	4/3	41	37
5527	5511	10	2 ^c	4/6	7	7		10	4/6	4/4		8	4/4	38	36
5528	5512	13		5/8	8	8		13	5/8	4/4		8	4/4	40	40
5529	5512														
5530	5513	11		4/7	8	7		11	4/7	4/4		8	4/4	40	42
5531	5513	10	1	3/7	7	7		10	3/7	3/5		8	3/5	38	40
5532	5514	12		4/8	7	7		12	4/8	4/4		8	4/4	38	34
5533	5514	12		8/4	6	6		12	8/4	4/4		8	4/4	39	39
5534	5506	10		6/4	7	6		10	6/4	4/4		8	4/4	31	35
5535	5506	13		5/8	5	4	7	6	2/4	2/4		6	2/4	38	34
TOTAL		184	5	79/105			9	175	76/99	58/67	1	124	58/66		
MEAN		12			7	7		11				8		39	38
SD		2.0			0.7	0.9		2.2				0.6		4.1	3.8
SE		0.5			0.2	0.2		0.5				0.1		1.1	1.0
N		16						16				16			

^aHis-sexed pup, corrected at Day 21.^bHis-sexed pup, corrected at Day 4.^cSiamese birth, recorded as 2 male pups.

TABLE I-E-24

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 10

LITTER OBSERVATIONS

SECOND GENERATION - FIRST MATING (F2a)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATION</u>
0	5463	0	ONE FEMALE PUP - HEMATOMA, NOSE; AND TOP OF HEAD.
	5461	1	ONE MALE PUP AND ONE FEMALE PUP - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5467	1	SURVIVING PUPS - TWO MALE AND ONE FEMALE - DRY SPLITTING SKIN AROUND AXILLA AND VENTRAL SIDE OF NECK.
	5474	1	ONE MALE PUP - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5458	21	ONE MALE PUP - FOUND DEAD; NO VISIBLE ABNORMALITIES.
300	5503	0	ONE FEMALE PUP - HEMATOMA, RIGHT SIDE OF FACE.
	5495	2	ONE FEMALE PUP - FOUND DEAD; NO VISIBLE ABNORMALITIES.
3000	5527	0	TWO MALE PUPS CONNECTED AT VENTRAL THORACIC REGION - FOUND DEAD; EACH HAS A COMPLETE SET OF LIMBS, ONE HAS COMPLETELY FORMED HEAD AND FACE, THE OTHER HAS ONLY TWO PROTRUSIONS FROM THE ANTERIOR NECK.
	5533	2	ONE PUP - FOUND DEAD; UNABLE TO DETERMINE SEX DUE TO CAN- NIBALIZATION.
	5526	21	ONE MALE AND TWO FEMALE PUPS - BOTH EYES CLOSED.
	5527	21	THREE MALE AND THREE FEMALE PUPS - PARTIALLY CLOSED EYES
	5534	26	ONE MALE PUP - VERY EMACIATED AND SMALL, RIGHT EYE CLOSED

TABLE I-E-25

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 11

PUP NECROPSY OBSERVATIONS (F2a) AT DAY 21

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>NUMBER OF PUPS</u>	<u>OBSERVATION</u>
0	5456	8	ALL TISSUES APPEAR NORMAL
	5458	6	ALL TISSUES APPEAR NORMAL
	5462	8	ALL TISSUES APPEAR NORMAL
	5463	8	ALL TISSUES APPEAR NORMAL
	5466	8	ALL TISSUES APPEAR NORMAL
	5470	8	ALL TISSUES APPEAR NORMAL
300	5490	8	ALL TISSUES APPEAR NORMAL
	5491	8	ALL TISSUES APPEAR NORMAL
	5494	8	ALL TISSUES APPEAR NORMAL
	5496	8	ALL TISSUES APPEAR NORMAL
	5499	6	ALL TISSUES APPEAR NORMAL
	5502	7	ALL TISSUES APPEAR NORMAL
3000	5520	8	ALL TISSUES APPEAR NORMAL
	5523	7	ALL TISSUES APPEAR NORMAL
	5524	8	ALL TISSUES APPEAR NORMAL
	5530	8	ALL TISSUES APPEAR NORMAL
	5533	8	ALL TISSUES APPEAR NORMAL
	5534	8	ALL TISSUES APPEAR NORMAL

TABLE 1-E-26

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 12

RESULTS OF SECOND GENERATION - SECOND MATING (F2b)

DOSE - 0 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				LITTER REDUCED		DAY 21			
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	M/F	M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT
5456	5455	12		6/6	6	5		12	6/6	4/4		8	4/4	44	46
5457	5455	15		8/7	6	6		15	8/7	4/4		8	4/4	41	40
5458	5454	14		5/9	7	6		14	5/9	4/4		8	4/4	46	46
5459	5454	16		7/9	6	6		16	7/9	4/4		8	4/4	42	41
5460	5453	11		3/8	6	6		11	3/8	3/5		8	3/5	38	35
5461	5453	16		8/8	6	5	1	15	8/7	4/4		8	4/4	40	37
5462	5452	12		7/5	7	7		12	7/5	4/4		8	4/4	54	50
5463	5452	14		8/6 ^a	6	6		14	9/5	4/4		8	4/4	46	48
5464	5451														
5465	5451	15		9/6	6	6		15	9/6	4/4		8	4/4	46	40
5466	5450	12		7/5	6	6	1	11	6/5	4/4		8	4/4	38	35
5467	5450														
5468	5449	8	2	1/7	6	7		8	1/7	1/7		8	1/7	47	48
5469	5449	15		6/9	7	7		15	6/9	4/4		8	4/4	50	45
5470	5448	17	1	9/8	7	6		17	9/8	4/4		7	4/3	51	49
5471	5448	13	1	8/5	6	5	5	8	7/1	7/1		7	6/1	44	36
5472	5447	13	1	5/8	8	7		13	5/8	4/4		8	4/4	52	48
5473	5447	16		7/9	6	6		16	7/9	4/4		8	4/4	36	33
5474	5446	13		6/7	6	6		13	6/7	4/4		8	4/4	46	44
5475	5446	18	1	9/9	6	6	1	17	9/8	4/4		8	4/4	39	41
TOTAL		250	6	119/131			8	242	118/124	71/73	2	142	70/72		
MEAN		14			6	6		13				8		44	42
SD		2.4			0.6	0.6		2.7				0.3		5.5	5.4
SE		0.6			0.2	0.2		0.6				0.1		1.4	1.4
N		18						18				18			

^aThis-corrected at Day 0, corrected at Day 4.

TABLE I-E-26 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-06

TABLE 12 (Continued)

RESULTS OF SECOND GENERATION - SECOND MATING (F2b)

DOSE - 300 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5486	5485	16		8/8	6	6	1	15	7/8	4/4		8	4/4	46	46
5487	5485	13		2/11 ^a	6	7		13	3/10	3/5		8	3/5	52	47
5488	5484	12		7/5	7	7	1	11	7/4	4/4		8	4/4	50	48
5489	5484														
5490	5483	13		8/5 ^a	7	6		13	7/6	4/4	1	7	4/3	55	50
5491	5483	8		8/0	7	8		8	8/0	8/0		8	8/0	46	
5492	5482	12		6/6	7	8		12	6/6	4/4		8	4/4	39	42
5493	5482	18		8/10	8	7		18	8/10	4/4		8	4/4	54	49
5494	5481	15	2	5/10	6	6	1	14	5/9	4/4		8	4/4	51	50
5495	5481	17		8/9 ^a	6	6		17	7/10	4/4		8	4/4	49	49
5496	5480	16		7/9	6	6		16	7/9	4/4		8	4/4	39	41
5497	5480	17		10/7	9	9		17	10/7	4/4		8	4/4	36	33
5498	5479	16		8/8	6	5	2	14	8/6	4/4		8	4/4	44	40
5499	5479														
5500	5478	10		9/1		7		10	9/1	7/1		8	7/1	56	44
5501	5478	9		5/4	6	6		9	5/4	4/4		8	4/4	54	49
5502	5477	9		4/5 ^a	6	7		9	5/4	4/4		8	3/5	47	43
5503	5477	12		6/6	6	6		12	6/6	4/4		8	4/4	44	40
5504	5476	15		8/7	6	6	1	14	7/7	4/4		8	4/4	43	46
5505	5476														
TOTAL		228	2	117/111			6	222	115/107	74/62	1	135	73/62		
MEAN		13			7	7		13				8		47	44
SD		3.1			1.0	1.0		3.0				0.2		6.3	5.0
SE		0.8			0.2	0.3		0.7				0.1		1.7	1.4
N		17						17				17			

^a Mis-sexed at Day 0, corrected at Day 4.

TABLE I-E-26 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 12 (Continued)

RESULTS OF SECOND GENERATION - SECOND MATING (F2b)

DOSE - 3000 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
5516	5515	12		6/6	6	6		12	6/6	4/4		8	4/4	56	49
5517	5515														
5518	5514	12		10/2	7	7		12	10/2	6/2		8	6/2	51	51
5519	5514	14		9/5	7	7		14	9/5	4/4		8	4/4	46	45
5520	5513	14		8/6 ^a	6	6		14	6/8	4/4		8	4/4	42	39
5521	5513	14		6/8	8	7		14	6/8	4/4		8	4/4	68	62
5522	5512	7		5/2	8	7	1	6	5/1	5/1		6	5/1	57	50
5523	5512	7		3/4	6	5		7	3/4	3/4		7	3/4	30	31
5524	5511	14		8/6	6	6		14	8/6	4/4		8	4/4	43	44
5525	5511	14		4/10 ^a	6	6		14	5/9	4/4		8	4/4	46	43
5526	5510	11		6/5	7	7		11	6/5	4/4		8	4/4	51	44
5527	5510	14		8/6	6	6		14	8/6	4/4		8	4/4	44	42
5528	5509														
5529	5509	13		4/9	8	7		13	4/9	4/4		8	4/4	49	49
5530	5508	15		5/10	7	6		15	5/10	4/4		8	4/4	51	47
5531	5508	13		8/5	6	6		13	8/5	4/4		8	4/4	44	44
5532	5507	14		4/10	8	7	1	13	4/9	4/4		8	4/4	38	36
5533	5507	16		10/6	6	7		16	10/6	4/4		8	5/3	46	45
5534	5506	13	2	7/6	6	6		13	7/6	4/4		8	4/4	50	46
5535	5506	16		6/10	6	6	1	15	6/9	4/4	1	7	3/4	54	50
TOTAL		233	2	117/116	7	6	3	230	116/114	74/67	1	140	74/66	48	45
MEAN		13			0.8	0.6		13				8		8.7	7.0
SD		2.5			0.2	0.1		2.6				0.5		2.2	1.7
SE		0.6						0.6				0.1			
N		18						18				18			

^aMis-sexed at Day 0, corrected at Day 4.

TABLE I-E-27

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 13

LITTER OBSERVATIONS

SECOND GENERATION - SECOND MATING (F2b)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATION</u>
0	5471	0	ONE MALE PUP - PALE AND INACTIVE; DEATH RECORDED SUBSEQUENTLY.
	5471	21	ONE PUP - FOUND DEAD; CANNIBALIZED.
	5472	0	ONE FEMALE PUP - HEMATOMA, MID- DORSAL THORACIC REGION.
300	5504	1	ONE MALE PUP - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5494	4	ONE FEMALE PUP - FOUND DEAD.
	5497	6	ONE FEMALE PUP - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5490	9	ONE FEMALE PUP - FOUND DEAD.
3000	5524	21	ONE FEMALE PUP - ACCIDENTALLY DIED DURING HANDLING.

TABLE I-E-28

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 14

BODY WEIGHTS AND FOOD CONSUMPTION (G) OF PARENT RATS (F1b)

BODY WEIGHTS

DOSE (PPM)		MALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	341	464	468	578
	SD	27.6	30.4	37.2	45.3
	SE	8.7	9.6	11.8	14.3
	N	10	10	10	10
300	MEAN	313	445	451	572
	SD	32.2	22.2	27.5	41.4
	SE	10.2	7.0	8.7	13.1
	N	10	10	10	10
3000	MEAN	310*	438	442	541
	SD	27.3	31.5	33.7	38.6
	SE	8.6	10.0	10.7	12.2
	N	10	10	10	10

FOOD CONSUMPTION

DOSE (PPM)		MALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	24.3	25.3	26.0	32.0
	SD	2.2	1.4	2.5	5.4
	SE	0.7	0.6	1.0	1.8
	N	9	5	6	9
300	MEAN	24.0	25.9	23.9	31.7
	SD	1.8	2.9	6.1	3.9
	SE	0.6	1.2	2.1	1.2
	N	9	6	8	10
3000	MEAN	24.6	27.3	27.9	28.6
	SD	2.1	2.4	2.5	4.2
	SE	0.7	0.9	0.9	1.4
	N	9	7	8	9

*p < 0.05 as compared to controls: Dunnett's t-test.

TABLE I-E-28 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 14 (Continued)

BODY WEIGHTS AND FOOD CONSUMPTION (G) OF PARENT RATS (F1b)

BODY WEIGHTS

DOSE (PPM)		FEMALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	200	246	249	295
	SD	20.9	18.6	22.0	16.6
	SE	4.7	4.2	4.9	3.7
	N	20	20	20	20
300	MEAN	203	261	259	308
	SD	24.0	25.2	24.3	26.5
	SE	5.4	5.6	5.4	5.9
	N	20	20	20	20
3000	MEAN	194	247	243	287
	SD	18.6	22.3	22.0	23.3
	SE	4.2	5.0	4.9	5.2
	N	20	20	20	20

FOOD CONSUMPTION

DOSE (PPM)		FEMALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	17.4	23.1	22.7	31.0
	SD	3.7	4.7	4.9	4.7
	SE	0.8	1.2	1.1	1.3
	N	19	17	20	14
300	MEAN	18.8	21.9	22.5	31.4
	SD	2.7	4.1	4.2	5.7
	SE	0.6	1.0	1.0	1.3
	N	20	18	19	18
3000	MEAN	17.2	24.2	23.1	31.4
	SD	2.7	3.1	3.4	6.1
	SE	0.6	0.7	0.7	1.4
	N	18	17	20	20

TABLE 15

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NUMBER - 10734-06

WEEK NO. ** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
-001 CONTROL	MALE 05446 *****	STARTED OCT. 20, 1977
001 CONTROL	MALE 05446	ALL ANIMALS NORMAL
002 CONTROL	MALE 05446	ALL ANIMALS NORMAL
003 CONTROL	MALE 05446	ALL ANIMALS NORMAL
004 CONTROL	MALE 05446	ALL ANIMALS NORMAL
005 CONTROL	MALE 05446	ALL ANIMALS NORMAL
006 CONTROL	MALE 05446	ALL ANIMALS NORMAL
007 CONTROL	MALE 05446	ALL ANIMALS NORMAL
008 CONTROL	MALE 05446	ALL ANIMALS APPEAR NORMAL
009 CONTROL 3000 PPM	MALE 05446 FEMALE 05524 *****	ALL ANIMALS NORMAL ULCERATION : HEAD RIGHT SIDE LOSS OF : EAR-RIGHT
010 3000 PPM	FEMALE 05524 *****	MEDIUM ULCERATION(1-5CM) : EAR-RIGHT MEDIUM SCAB(1-5CM) : EAR-RIGHT RT EAR MISSING; SCAB ON RT SIDE OF HEAD LOSS OF : EAR-RIGHT
011 3000 PPM	FEMALE 05524	MEDIUM ULCERATION(1-5CM) : EAR-RIGHT LOSS OF : EAR-RIGHT MATE TO GET F2A
012 300 PPM 3000 PPM	FEMALE 05489 05524 *****	LOCAL HAIR LOSS : LIMBS-FORE MEDIUM SCAB(1-5CM) : EAR-RIGHT RIGHT EAR MISSING SCAB ON RT SIDE OF HEAD LOSS OF : EAR-RIGHT
013 CONTROL 300 PPM 3000 PPM	MALE 05446 FEMALE 05489 05524	REMOVED FROM MATING LOCAL HAIR LOSS : LIMBS-FORE LOSS OF : EAR-RIGHT
014 300 PPM	FEMALE 05489	LOCAL HAIR LOSS : LIMBS-FORE

TABLE I-E-29 (Continued)

TABLE 15 (CONTINUED)

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NO. - 10734-06

WEEK NO.	** DOSE GROUP/SEX	* ANIMAL NUMBER	***** OBSERVATIONS : QUALIFIER COMMENTS	*****
	3000 PPM	05524	LOSS OF : EAR-RIGHT	
015	300 PPM 3000 PPM	FEMALE 05489 05524	LOCAL HAIR LOSS : LIMBS-FORE LOSS OF : EAR-RIGHT	
016	300 PPM 3000 PPM	FEMALE 05489 05524	LOCAL HAIR LOSS : LIMBS-FORE LOSS OF : EAR-RIGHT	
017	300 PPM	FEMALE 05489	LOCAL HAIR LOSS : LIMBS-FORE	
018	300 PPM 3000 PPM	FEMALE 05489 05524	LOCAL HAIR LOSS : LIMBS-FORE LOSS OF : EAR-RIGHT	
020	300 PPM 3000 PPM	FEMALE 05489 05524	LOCAL HAIR LOSS : LIMBS-FORE LOSS OF : EAR-RIGHT	
021	CONTROL 300 PPM	MALE 05446 FEMALE 05489	MATE TO GET F2B LOCAL HAIR LOSS : LIMBS-FORE	
022	300 PPM	FEMALE 05489	LOCAL HAIR LOSS : LIMBS-FORE	
023	CONTROL 300 PPM	MALE 05446 FEMALE 05489	REMOVED FROM MATING LOCAL HAIR LOSS : LIMBS-FORE	
024	300 PPM	FEMALE 05489	LOCAL HAIR LOSS : LIMBS-FORE LOCAL HAIR LOSS : LIMBS-FORE	
025	CONTROL 300 PPM	FEMALE 05468 05489	EYE DISCHARGE : EYE-LEFT LOCAL HAIR LOSS : LIMBS-FORE	
026	300 PPM	FEMALE 05489	LOCAL HAIR LOSS : LIMBS-FORE	
027	CONTROL	MALE 05446 FEMALE 05456	ALL MALES SACRIFICED-GROSS NECROPSY ALL TISSUES NORMAL ALL FEMALES SACRIFICED ON OR BEFORE WEEK 29 GROSS NECROPSY-ABNORMALS LISTED	
	300 PPM	05489	LOCAL HAIR LOSS : LIMBS-FORE	
028	300 PPM	FEMALE 05489	LOCAL HAIR LOSS : LIMBS-FORE	

TABLE I-E-30

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 16

PARENT NECROPSY OBSERVATIONS (F1b) - MALES

DOSE (PPM)	MALE NUMBER	OBSERVATION
0	5446	ALL TISSUES APPEAR NORMAL
	5447	ALL TISSUES APPEAR NORMAL
	5448	ALL TISSUES APPEAR NORMAL
	5449	ALL TISSUES APPEAR NORMAL
	5450	ALL TISSUES APPEAR NORMAL
	5451	ALL TISSUES APPEAR NORMAL
	5452	ALL TISSUES APPEAR NORMAL
	5453	ALL TISSUES APPEAR NORMAL
	5454	ALL TISSUES APPEAR NORMAL
	5455	ALL TISSUES APPEAR NORMAL
300	5476	ALL TISSUES APPEAR NORMAL
	5477	ALL TISSUES APPEAR NORMAL
	5478	ALL TISSUES APPEAR NORMAL
	5479	ALL TISSUES APPEAR NORMAL
	5480	ALL TISSUES APPEAR NORMAL
	5481	ALL TISSUES APPEAR NORMAL
	5482	ALL TISSUES APPEAR NORMAL
	5483	ALL TISSUES APPEAR NORMAL
	5484	ALL TISSUES APPEAR NORMAL
	5485	ALL TISSUES APPEAR NORMAL
3000	5506	ALL TISSUES APPEAR NORMAL
	5507	ALL TISSUES APPEAR NORMAL
	5508	ALL TISSUES APPEAR NORMAL
	5509	ALL TISSUES APPEAR NORMAL
	5510	ALL TISSUES APPEAR NORMAL
	5511	ALL TISSUES APPEAR NORMAL
	5512	ALL TISSUES APPEAR NORMAL
	5513	ALL TISSUES APPEAR NORMAL
	5514	ALL TISSUES APPEAR NORMAL
	5515	ALL TISSUES APPEAR NORMAL

TABLE I-E-30 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 16 (Continued)

PARENT NECROPSY OBSERVATIONS (F1b) - FEMALES

<u>DOSE</u> <u>(PPM)</u>	<u>FEMALE</u> <u>NUMBER</u>	<u>OBSERVATION</u>
0	5456	ALL TISSUES APPEAR NORMAL
	5457	CYST ON RIGHT OVARY (10 X 15 X 10 MM)
	5458	CYST ON UTERUS (13 X 10 MM)
	5459	ALL TISSUES APPEAR NORMAL
	5460	ALL TISSUES APPEAR NORMAL
	5461	ALL TISSUES APPEAR NORMAL
	5462	ALL TISSUES APPEAR NORMAL
	5463	ALL TISSUES APPEAR NORMAL
	5464	ALL TISSUES APPEAR NORMAL
	5465	ALL TISSUES APPEAR NORMAL
	5466	ALL TISSUES APPEAR NORMAL
	5467	ALL TISSUES APPEAR NORMAL
	5468	ALL TISSUES APPEAR NORMAL
	5469	ALL TISSUES APPEAR NORMAL
	5470	ALL TISSUES APPEAR NORMAL
	5471	ALL TISSUES APPEAR NORMAL
	5472	ALL TISSUES APPEAR NORMAL
	5473	ALL TISSUES APPEAR NORMAL
	5474	ALL TISSUES APPEAR NORMAL
	5475	ALL TISSUES APPEAR NORMAL
300	5486	ALL TISSUES APPEAR NORMAL
	5487	ALL TISSUES APPEAR NORMAL
	5488	ALL TISSUES APPEAR NORMAL
	5489	ALL TISSUES APPEAR NORMAL
	5490	SMALL CYST ON RIGHT OVARY; ALL OTHER TISSUES APPEAR NORMAL
	5491	ALL TISSUES APPEAR NORMAL
	5492	ALL TISSUES APPEAR NORMAL
	5493	ALL TISSUES APPEAR NORMAL
	5494	ALL TISSUES APPEAR NORMAL
	5495	ALL TISSUES APPEAR NORMAL
	5496	ALL TISSUES APPEAR NORMAL
	5497	ALL TISSUES APPEAR NORMAL
	5498	ALL TISSUES APPEAR NORMAL
	5499	ALL TISSUES APPEAR NORMAL
	5500	ALL TISSUES APPEAR NORMAL
	5501	ALL TISSUES APPEAR NORMAL
	5502	ALL TISSUES APPEAR NORMAL
	5503	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	5504	ALL TISSUES APPEAR NORMAL
	5505	ALL TISSUES APPEAR NORMAL

TABLE I-E-30 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 16 (Continued)

PARENT NECROPSY OBSERVATIONS (F1b) - FEMALES

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>OBSERVATION</u>
3000	5516	ALL TISSUES APPEAR NORMAL
	5517	ALL TISSUES APPEAR NORMAL
	5518	ALL TISSUES APPEAR NORMAL
	5519	ALL TISSUES APPEAR NORMAL
	5520	ALL TISSUES APPEAR NORMAL
	5521	ALL TISSUES APPEAR NORMAL
	5522	ALL TISSUES APPEAR NORMAL
	5523	FOUR STONES IN URINARY BLADDER, EACH APPROXIMATELY 1 CM ³
	5524	ALL TISSUES APPEAR NORMAL
	5525	ALL TISSUES APPEAR NORMAL
	5526	ALL TISSUES APPEAR NORMAL
	5527	ALL TISSUES APPEAR NORMAL
	5528	ALL TISSUES APPEAR NORMAL
	5529	ALL TISSUES APPEAR NORMAL
	5530	CYST ON RIGHT FALLOPIAN TUBE NEAR UTERUS (5 X 5 X 5 MM)
	5531	ALL TISSUES APPEAR NORMAL
	5532	ALL TISSUES APPEAR NORMAL
	5534	ALL TISSUES APPEAR NORMAL
	5535	ALL TISSUES APPEAR NORMAL

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-06

TABLE 17

RESULTS OF THIRD GENERATION - FIRST MATING (F3a)

DOSE - 0 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
1407	1405	13		8/5	7	6		13	8/5	4/4		8	4/4	34	33
1408	1406	13	1	6/7	6	5	1	12	5/7	4/4		8	4/4	39	36
1409	1406	16		10/6	6	6		16	10/6	4/4 ^a		8	5/3	38	44
1397	1410	15		5/10	6	5		15	5/10	4/4		8	4/4	47	45
1411	1397	13		7/6	6	6		13	7/6	4/4		8	4/4	40	36
1412	1398	6		4/2	7	7		6	4/2	4/2		6	4/2	46	46
1413	1398														
1414	1399	14		10/4	6	6	1	13	9/4	4/4		8	4/4	45	42
1415	1399	12		5/7	8	8		12	5/7	4/4		8	4/4	46	40
1416	1400	12	1	6/6	7	6		12	6/6	4/4		8	4/4	41	38
1417	1400	11		5/6	6	6		11	5/6	4/4		8	4/4	43	42
1418	1401														
1419	1401	9		5/4	7	6		9	5/4	4/4		8	4/4	56	49
1420	1402	5		1/4	4	4	5	0							
1421	1402	14		8/6	6	7		14	8/6	4/4		8	4/4	41	40
1422	1403	14		8/6	6	6	1	13	8/5	4/4		8	4/4	50	47
1423	1403	15		8/7	6	6		15	8/7	4/4		8	4/4	45	43
1424	1404	11		6/5	7	6		11	6/5	4/4		7	3/4	33	33
1425	1404	3		0/3	8	8		3	0/3	0/3	1	3	0/3	58	58
1426	1405	14		6/8	7	6		14	6/8	4/4		8	4/4	34	28
TOTAL		210	2	108/102			8	202	105/97	64/65	1	128	64/64		
MEAN		12			6	6		12				8		42	41
SD		3.7			0.9	1.0		3.3				1.3		6.2	7.1
SE		0.9			0.2	0.2		0.8				0.3		1.6	1.7
N		18						17				17			

^aOne pup mis-sexed, corrected at Day 21.

TABLE I-E-31 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 17 (Continued)

RESULTS OF THIRD GENERATION - FIRST MATING (F3a)

DOSE - 300 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21		
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F
1437	1428	12		8/4	5	5	3	9	6/3	5/3
1438	1428	14		9/5	5	5	3	11	6/5	4/4
1439	1429									
1440	1429	16		13/3	6	6		16	13/3	5/3
1441	1430	13		10/3	6	6	1	12	9/3	5/3
1442	1430	11		9/2	6	6	1	10	9/1	7/1
1443	1431	12		5/7	7	6		12	5/7	4/4
1444	1431	11		6/5	7	7		11	6/5	4/4
1445	1432	14		11/3	6	6		14	11/3	5/3
1446	1432									
1447	1433	13		9/4	7	7		13	9/4	4/4
1448	1433	7		4/3	7	7		7	4/3	4/3
1449	1434	22		11/11	7	7		22	11/11	4/4
1450	1434	12		8/4	6	5		12	8/4	4/4
1451	1435	12		8/4	6	7		12	8/4	4/4
1452	1435	13		6/7	7	6		13	6/7	4/4
1453	1436									
1454	1436	2		0/2	7	6		2	0/2	0/2
1455	1427	13		4/9	7	6		13	4/9	4/4
1456	1427	15		6/9	7	7		15	6/9	4/4
TOTAL		212		127/85	6	6	8	204	121/83	71/58
MEAN		13			0.7	0.8		13		
SD		4.0			0.2	0.2		3.3		
SE		1.0						0.8		
N		17						16		

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 17 (Continued)

RESULTS OF THIRD GENERATION - FIRST MATING (F3a)

NOISE - 3000 RPM

	FEMALE NUMBER	DAY 0	DAY 4						DAY 21							
			LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
1467	1465	17			10/7	6	6		17	10/7	4/4		8	4/4	33	32
1468	1466	16	1		10/6	6	6		16	10/6	4/4		7	4/3	32	33
1469	1466	13			7/6	6	6	1	12	7/5	4/4		7	3/4	33	31
1470	1457	5			4/1	7	7		5	4/1	4/1		5	4/1	31	29
1471	1457	14			9/5	6	6		14	9/5	4/4		6	2/4	26	28
1472	1458	13			5/8	7	6		13	5/8	4/4		8	4/4	33	33
1473	1458	13			8/5	6	6		13	8/5	4/4		8	4/4	31	31
1474	1459															
1475	1459															
1476	1460	10			4/6	7	7		10	4/6	4/4		8	4/4	20	21
1477	1460	8			4/4	8	8		8	4/4	4/4		7	3/4	31	30
1478	1461	12			4/8	7	7		12	4/8	4/4					
1479	1461	11			6/5	4	4		11	6/5	4/4		8	4/4	30	30
1480	1462	13			8/5	7	7		13	8/5	4/4		8	4/4	28	26
1481	1462	14			11/3	6	5		14	11/3	5/3		3	2/1	26	14
1482	1463	5		1	3/2	9	9		5	3/2	3/2		5	3/2	58	55
1483	1463	15			8/7	6	6		15	8/7	4/4		6	2/4	27	26
1484	1464	14			9/6	7	7		14	8/6	4/4		4	2/2	34	28
1485	1464	14			8/5	7	6		14	9/5	4/4		8	4/4	31	30
1486	1465	12			6/6	6	6	1	11	5/6	4/4		2	0/2		26
TOTAL		219	2		124/95			2	217	123/94	72/66		108	53/55		
MEAN		12				7	6		12				6		32	30
SD		3.3				1.0	1.1		3.3				1.9		7.9	8.1
SE		0.8				0.2	0.3		0.8				0.5		2.0	2.0
n		18							18				17			

TABLE I-E-32

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 18

LITTER OBSERVATIONS

THIRD GENERATION - FIRST MATING (F3a)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATION</u>
300	1438	20	ONE MALE AND TWO FEMALE PUPS - FOUND DEAD; ONE PUP - CANNI- BALIZED.
3000	1478	9	ALL PUPS (EIGHT) - FOUND DEAD.
	1484	14	THREE FEMALE PUPS - FOUND DEAD; ONE PUP - CANNIBALIZED.
	1469	17	ONE MALE PUP - FOUND DEAD.
	1477	19	ONE MALE PUP - FOUND DEAD.
	1471	20	ONE MALE PUP - FOUND DEAD; ONE PUP - CANNIBALIZED.
	1486	20	TWO FEMALE AND TWO MALE PUPS - FOUND DEAD; ONE PUP - CANNIBALIZED.
	1481	21	FOUR FEMALE PUPS - FOUND DEAD; ONE PUP - CANNIBALIZED.
	1481	23	ONE PUP - CANNIBALIZED.

TABLE I-E-33

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 19

PUP NECROPSY OBSERVATIONS (F3a) AT DAY 21

<u>DOSE</u> <u>(PPM)</u>	<u>FEMALE</u> <u>NUMBER</u>	<u>NUMBER</u> <u>OF PUPS</u>	<u>OBSERVATION</u>
0	1408	8	ALL TISSUES APPEAR NORMAL
	1410	8	ALL TISSUES APPEAR NORMAL
	1415	8	ALL TISSUES APPEAR NORMAL
	1423	8	ALL TISSUES APPEAR NORMAL
	1424	7	ALL TISSUES APPEAR NORMAL
	1426	8	ALL TISSUES APPEAR NORMAL
300	1437	8	ALL TISSUES APPEAR NORMAL
	1438	4	ALL TISSUES APPEAR NORMAL
	1440	8	ALL TISSUES APPEAR NORMAL
	1442	7	ALL TISSUES APPEAR NORMAL
	1449	8	ALL TISSUES APPEAR NORMAL
	1454	2	ALL TISSUES APPEAR NORMAL
3000	1468	7	ALL TISSUES APPEAR NORMAL
	1470	5	ALL TISSUES APPEAR NORMAL
	1476	8	ALL TISSUES APPEAR NORMAL
	1480	8	ALL TISSUES APPEAR NORMAL
	1484	4	ALL TISSUES APPEAR NORMAL
	1485	8	ALL TISSUES APPEAR NORMAL

TABLE I-E-34

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-06

TABLE 20

RESULTS OF THIRD GENERATION - SECOND MATING (F3b)

DOSE - 0 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21		
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F
1407	1402	17		5/12 ^a	5	5	2	15	3/12	3/5
1408	1405	7		5/2	5	5		7	5/2	4/2
1409	1405	20		13/7	6	5	1	19	12/7	4/4
1410	1406	16		8/8	6	6		16	8/8	4/4
1411	1406	17		12/5	6	6		17	12/5	4/4
1412	1397	11		11/0	7			11	11/0	8/0
1413	1397	13		8/5	5	5		13	8/5	4/4
1414	1398	18		12/6	6	6		18	12/6	4/4
1415	1398	12		6/6	7	7		12	6/6	4/4
1416	1399	15	1	10/5	6	5	1	14	9/5	4/4
1417	1399	15	1	13/2	6	5	3	12	10/2	6/2
1418	1400	13	1	6/7	6	6		13	6/7	4/4
1419	1400	16		4/12	5	5	1	15	4/11	4/4
1420	1401	7		5/2	7	6		7	5/2	5/2
1421	1401	15		5/10	6	5		15	5/10	4/4
1422	1402	18		10/8	6	6		18	10/8	4/4
1423	1403	18		9/9	6	5		18	9/9	4/4
1424	1403	12		7/5	7	6		12	7/5	4/4
1425	1404	13		2/13	5	5	2	13	7/6	4/4
1426	1404	14		3/11	5	5		14	3/11	3/5
TOTAL		287	3	159/128	6	5	10	279	152/127	85/72
MEAN		14			6	5		14		
SD		3.5			0.7	0.6		3.3		
SF		0.8			0.2	0.1		0.7		
H		20						20		

^apups initially mis-sexed.

TABLE I-E-34 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 20 (Continued)

RESULTS OF THIRD GENERATION - SECOND MATING (F3b)

DOSE - 300 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED M/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
1437	1436	9		4/5	7	6		9	4/5	4/4		8	4/4	49	49
1438	1436	6		2/4	7	7		6	2/4	2/4		6	2/4	49	44
1439	1428	12		5/7	6	6		12	5/7	4/4	2	6	3/3	46	42
1440	1428	13		7/6 ^a	5	6		13	7/6	4/4		8	4/4	36	36
1441	1429	13		4/9	6	6		13	4/9	4/4		8	4/4	54	53
1442	1429	16		8/8	6	5		16	8/8	4/4		8	4/4	50	45
1443	1430	14		11/3	7	6		14	11/3	5/3		8	5/3	51	48
1444	1430	15		9/6 ^a	5	5	1	14	8/6	4/4		8	4/4	51	40
1445	1431	7		5/2	7	7		7	5/2	5/2		7	5/2	46	39
1446	1432	14		10/4	6	4		14	10/4	4/4		8	4/4	38	31
1447	1432	17		10/7	7	6	1	16	10/6	4/4		8	4/4	53	42
1448	1433	15	1	6/9	7	6	3	12	4/8	4/4		8	4/4	48	45
1449	1433	20		10/10 ^a	6	6	1	19	10/9	4/4		8	4/4	53	46
1450	1434	13		10/3	6	6		13	10/3	5/3		8	4/4	48	51
1451	1434	17		8/9	6	6	1	16	7/9	4/4	2	6	2/4	40	34
1452	1434	11		4/7	6	6		11	4/7	4/4		8	4/4	49	44
1453	1435	10		5/5	6	6		10	5/5	4/4		8	4/4	49	44
1454	1427	17		8/9	7	5		17	8/9	4/4		8	4/4	50	40
1455	1427	17		10/7 ^a	5	5		17	10/7	4/4		8	4/4	41	34
1456	1435	256	1	136/120	6	6	7	249	132/117	77/72	4	145	73/72	47	43
TOTAL		13						13				8		5.1	5.9
MEAN		3.7			0.7	0.7		3.4				0.8		1.2	1.4
SD		0.8			0.2	0.2		0.8				0.2			
SE		19						19				19			

^apups initially mis-sexed.

TABLE I-E-34 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 20 (Continued)

RESULTS OF THIRD GENERATION - SECOND MATING (F3b)

DOSE - 3000 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0				DAY 4				DAY 21					
		LIVE PUPS	DEAD PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT	DEATHS DAY 1-4	LIVE PUPS	SEX M/F	LITTER REDUCED N/F	DEATHS DAY 4-21	LIVE PUPS	SEX M/F	MEAN M PUP WT	MEAN F PUP WT
1467	1466	16		8/8 ^a	6	6		16	8/8	4/4		8	4/4	52	45
1468	1457	15		9/6 ^a	7	7	1	14	8/6	4/4		8	4/4	47	44
1469	1457	12		7/5	7	7		12	7/5	4/4		8	4/4	45	36
1470	1458	12		3/9 ^a	6	5		12	3/9	3/5		8	3/5	48	43
1471	1458	18	1	8/10	6	5	1	17	8/9	4/4		8	4/4	47	37
1472	1459	6		3/3	8	7		6	3/3	3/3	6	0			
1473	1459	13		3/10 ^a	5	5		13	3/10	3/5		8	3/5	60	49
1474	1460														
1475	1460	15		6/9	6	5	15	9	3/6	3/5		8	3/5	46	39
1476	1461	11		4/7	7	5	2	12	4/8	4/4		8	4/4	46	40
1477	1461	12		4/8	6	6		12	6/6	4/4		8	4/4	39	36
1478	1562	12		6/6	7	7		12	6/6	4/4		8	4/4	41	35
1479	1462	17		10/7 ^a	6	6		17	10/7	4/4		6	4/4	42	41
1480	1466	13	3	4/9	5	5	6	7	5/2	5/2	1	6	4/2	46	42
1481	1463	17		9/8	6	5		17	9/8	4/4		8	4/4	46	42
1482	1464	10		4/6	6	5		10	4/6	4/4		8	4/4	39	37
1483	1464														
1484	1465	14		10/4	6	7		14	10/4	4/4		8	4/4	45	38
1485	1465	15		10/5	6	5		15	10/5	4/4		8	4/4	39	39
1486	1463	14		7/7	6	6		14	7/7	4/4		8	4/4	50	45
TOTAL		242	4	115/127	6	6	25	217	108/109	65/68	7	126	61/65	46	41
MEAN		13			6	6		13				7		5.4	4.5
SD		2.9			0.7	0.9		3.3				2.0		1.4	1.1
SE		0.7			0.2	0.2		0.8				0.5			
N		18						17				17			

^aPups initially mis-sexed.

TABLE I-E-35

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 21

LITTER OBSERVATIONS

THIRD GENERATION - SECOND MATING (F3b)

<u>DOSE</u> <u>(PPM)</u>	<u>FEMALE</u> <u>NUMBER</u>	<u>DAY OF</u> <u>LACTATION</u>	<u>OBSERVATION</u>
0	1414	4	ALL PUPS (EXCEPT ONE MALE PUP) - FOUND DEAD.
300	1453	2	ONE PUP - FOUND CANNIBALIZED.
	1452	16	TWO PUPS - FOUND CANNIBALIZED.
	1440	25	TWO PUPS (ONE CANNIBALIZED) - FOUND DEAD.
3000	1480	15	ONE PUP - FOUND CANNIBALIZED.

TABLE I-E-36

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 22

PUP NECROPSY OBSERVATIONS (F3b) AT DAY 21

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>NUMBER OF PUPS</u>	<u>OBSERVATION</u>
0	1408	6	ALL TISSUES APPEAR NORMAL
	1410	8	ALL TISSUES APPEAR NORMAL
	1411	8	ALL TISSUES APPEAR NORMAL
	1421	5	ALL TISSUES APPEAR NORMAL
	1422	8	ALL TISSUES APPEAR NORMAL
	1425	8	ALL TISSUES APPEAR NORMAL
300	1439	6	ALL TISSUES APPEAR NORMAL
	1440	6	ALL TISSUES APPEAR NORMAL
	1443	8	ALL TISSUES APPEAR NORMAL
	1447	8	ALL TISSUES APPEAR NORMAL
	1453	8	ALL TISSUES APPEAR NORMAL
	1455	8	ALL TISSUES APPEAR NORMAL
3000	1471	8	ALL TISSUES APPEAR NORMAL
	1477	8	ALL TISSUES APPEAR NORMAL
	1478	8	ALL TISSUES APPEAR NORMAL
	1482	8	ALL TISSUES APPEAR NORMAL
	1484	8	ALL TISSUES APPEAR NORMAL
	1485	8	ALL TISSUES APPEAR NORMAL

TABLE I-E-37

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 23

BODY WEIGHTS AND FOOD CONSUMPTION (G) OF PARENT RATS (F2b)

BODY WEIGHTS

DOSE (PPM)		MALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	338	481	504	564
	SD	26.1	40.7	41.2	43.6
	SE	8.2	12.9	13.0	13.8
	N	10	10	10	10
300	MEAN	328	441	482	553
	SD	19.1	30.7	41.1	51.0
	SE	6.0	9.7	13.0	16.1
	N	10	10	10	10
3000	MEAN	328	449	480	538
	SD	32.0	45.9	49.7	68.2
	SE	10.1	14.5	15.7	21.6
	N	10	10	10	10

FOOD CONSUMPTION

DOSE (PPM)		MALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	38.6	33.7	30.4	29.4
	SD	2.4	4.3	3.9	3.6
	SE	0.9	1.3	1.2	1.1
	N	7	10	10	10
300	MEAN	39.1	31.4	29.3	29.2
	SD	3.6	2.4	2.8	3.9
	SE	1.3	0.9	0.9	1.2
	N	8	8	10	10
3000	MEAN	38.2	31.2	28.8	28.9
	SD	3.8	4.4	3.7	4.0
	SE	1.3	1.4	1.2	1.3
	N	9	10	10	10

TABLE I-E-37 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 23 (Continued)

BODY WEIGHTS AND FOOD CONSUMPTION (G) OF PARENT RATS (F2b)

BODY WEIGHTS

DOSE (PPM)		FEMALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	212	264	278	300
	SD	19.7	24.9	26.6	23.5
	SE	4.4	5.6	6.0	5.2
	N	20	20	20	20
300	MEAN	210	255	269	297
	SD	31.2	17.5	17.8	20.2
	SE	7.0	3.9	4.0	4.5
	N	20	20	20	20
3000	MEAN	204	250	265	281*
	SD	18.9	22.2	24.4	23.7
	SE	4.3	5.0	5.5	5.3
	N	19	20	20	20

FOOD CONSUMPTION

DOSE (PPM)		FEMALES			
		WK 4	WK 9	WK 11	WK 20
0	MEAN	34.8	29.8	29.3	30.0
	SD	5.1	4.2	6.7	2.2
	SE	1.1	1.0	1.5	0.6
	N	20	18	20	15
300	MEAN	33.2	25.4	25.9	29.5
	SD	5.8	5.5	6.8	6.4
	SE	1.3	1.3	1.5	1.5
	N	19	19	20	18
3000	MEAN	33.6	27.4	25.4	29.2
	SD	6.4	6.0	5.9	4.4
	SE	1.6	1.5	1.4	1.1
	N	16	17	18	16

* $p < 0.05$ as compared to controls: Dunnett's t-test.

TABLE I-E-38

TABLE 24

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NUMBER - 10734-06

WEEK NO. ** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
-001 CONTROL	MALE 01397 *****	STARTED MAY 11, 1978
001 CONTROL	MALE 01397	ALL ANIMALS NORMAL
002 CONTROL	MALE 01397	ALL ANIMALS NORMAL
003 CONTROL	MALE 01397	ALL ANIMALS NORMAL
004 CONTROL	MALE 01402	MISSING FOUND-RETURNED TO STUDY
005 CONTROL	MALE 01397	ALL ANIMALS NORMAL
006 CONTROL	MALE 01397	ALL ANIMALS NORMAL
007 CONTROL	MALE 01397	ALL ANIMALS NORMAL
008 CONTROL	MALE 01397	ALL ANIMALS NORMAL
009 CONTROL	MALE 01397	ALL ANIMALS NORMAL
010 CONTROL	MALE 01397	ALL ANIMALS NORMAL
011 CONTROL	MALE 01397	ALL ANIMALS NORMAL
012 CONTROL	MALE 01397	MATE TO GET F3A ALL ANIMALS NORMAL
013 CONTROL	MALE 01397	ALL ANIMALS NORMAL
014 CONTROL	MALE 01397 FEMALE 01409	REMOVED FROM MATING LOCAL HAIR LOSS : LATERAL-RIGHT, POSTERIOR LOCAL HAIR LOSS : LATERAL-LEFT, POSTERIOR
015 CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
300 PPM	01444	SMALL MASS(<1CM) : VENTRAL-AXILLARY, LEFT
016 CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
300 PPM	01444	MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT

TABLE I-E-38 (Continued)

TABLE 24 (CONTINUED)

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NO. - 10734-06

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS ***** :
017	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
018	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
019	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
	3000 PPM	01477	MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
			LOCAL HAIR LOSS : DORSAL-LUMBAR, MID
020	CONTROL	MALE 01397	MATE TO GET F3B
		FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
	3000 PPM	01477	MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
			LOCAL HAIR LOSS : DORSAL-LUMBAR, MID
021	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
022	CONTROL	MALE 01397	REMOVED FROM MATING
		FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
023	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
024	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
025	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
026	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT
027	CONTROL	FEMALE 01409	LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC
	300 PPM	01444	LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC
			MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT

TABLE I-E-38 (Continued)

TABLE 24 (CONTINUED)

TOXICOLOGY OBSERVATION REPORT

CLINICAL SIGNS

PROJECT NO. - 10734-06

WEEK NO.	** DOSE	GROUP/SEX *	ANIMAL NUMBER	*****	OBSERVATIONS : QUALIFIER COMMENTS	*****
			01447		URINE STAIN ON COAT	
			01451		EMACIATED	
					NASAL DISCHARGE	
			*****		CRUSTY NOSE	
					URINE STAIN ON COAT	
			01453		URINE STAIN ON COAT	
028	CONTROL	FEMALE	01409		LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC	
					LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC	
	300 PPM		01422		URINE STAIN ON COAT	
			01444		MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT	
			01451		EMACIATED	
029	CONTROL	FEMALE	01409		LOCAL HAIR LOSS : LATERAL-RIGHT, THORACIC	
					LOCAL HAIR LOSS : LATERAL-LEFT, THORACIC	
	300 PPM		01422		URINE STAIN ON COAT	
			01444		MEDIUM MASS(1-5CM) : VENTRAL-AXILLARY, LEFT	
			01451		EMACIATED	

TABLE I-E-39

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 25

PARENT NECROPSY OBSERVATIONS (F2b) - MALES

<u>DOSE (PPM)</u>	<u>MALE NUMBER</u>	<u>OBSERVATION</u>
0	1397	ALL TISSUES APPEAR NORMAL
	1398	ALL TISSUES APPEAR NORMAL
	1399	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1400	ALL TISSUES APPEAR NORMAL
	1401	ALL TISSUES APPEAR NORMAL
	1402	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1403	ALL TISSUES APPEAR NORMAL
	1404	ALL TISSUES APPEAR NORMAL
	1405	ALL TISSUES APPEAR NORMAL
	1406	ALL TISSUES APPEAR NORMAL
300	1427	ALL TISSUES APPEAR NORMAL
	1428	ALL TISSUES APPEAR NORMAL
	1429	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1430	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1431	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1432	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1433	ALL TISSUES APPEAR NORMAL
	1434	ALL TISSUES APPEAR NORMAL
	1435	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1436	ALL TISSUES APPEAR NORMAL
3000	1457	ALL TISSUES APPEAR NORMAL
	1458	ALL TISSUES APPEAR NORMAL
	1459	ALL TISSUES APPEAR NORMAL
	1460	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1461	ALL TISSUES APPEAR NORMAL
	1462	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1463	ALL TISSUES APPEAR NORMAL
	1464	ALL TISSUES APPEAR NORMAL
	1465	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1466	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL

TABLE I-E-39 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 25 (Continued)

PARENT NECROPSY OBSERVATIONS (F2b) - FEMALES

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>OBSERVATION</u>
0	1407	ALL TISSUES APPEAR NORMAL
	1408	ALL TISSUES APPEAR NORMAL
	1409	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1410	ALL TISSUES APPEAR NORMAL
	1411	ALL TISSUES APPEAR NORMAL
	1412	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1413	ALL TISSUES APPEAR NORMAL
	1414	ALL TISSUES APPEAR NORMAL
	1415	ALL TISSUES APPEAR NORMAL
	1416	ALL TISSUES APPEAR NORMAL
	1417	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1418	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1419	ALL TISSUES APPEAR NORMAL
	1420	ALL TISSUES APPEAR NORMAL
	1421	ALL TISSUES APPEAR NORMAL
	1422	ALL TISSUES APPEAR NORMAL
	1423	ALL TISSUES APPEAR NORMAL
	1424	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1425	ALL TISSUES APPEAR NORMAL
	1426	ALL TISSUES APPEAR NORMAL
300	1437	ALL TISSUES APPEAR NORMAL
	1438	ALL TISSUES APPEAR NORMAL
	1439	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1440	ALL TISSUES APPEAR NORMAL
	1441	ALL TISSUES APPEAR NORMAL
	1442	ALL TISSUES APPEAR NORMAL
	1443	ALL TISSUES APPEAR NORMAL
	1444	BOTH KIDNEYS MOTTLED; SUBCUTANEOUS MAMMARY MASS (1 CM ²) ON LEFT AXILLA
	1445	ALL TISSUES APPEAR NORMAL
	1446	ALL TISSUES APPEAR NORMAL
	1447	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL

TABLE I-E-39 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-06

TABLE 25 (Continued)

PARENT NECROPSY OBSERVATIONS (F2b) - FEMALES

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>OBSERVATION</u>
300	1448	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1449	ALL TISSUES APPEAR NORMAL
	1450	ALL TISSUES APPEAR NORMAL
	1451	ALL TISSUES APPEAR NORMAL
	1452	ALL TISSUES APPEAR NORMAL
	1453	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1454	ALL TISSUES APPEAR NORMAL
	1455	ALL TISSUES APPEAR NORMAL
	1456	ALL TISSUES APPEAR NORMAL
3000	1467	ALL TISSUES APPEAR NORMAL
	1468	ALL TISSUES APPEAR NORMAL
	1469	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1470	ALL TISSUES APPEAR NORMAL
	1471	ALL TISSUES APPEAR NORMAL
	1472	ALL TISSUES APPEAR NORMAL
	1473	ALL TISSUES APPEAR NORMAL
	1474	ALL TISSUES APPEAR NORMAL
	1475	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1476	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1477	ALL TISSUES APPEAR NORMAL
	1478	ALL TISSUES APPEAR NORMAL
	1479	ALL TISSUES APPEAR NORMAL
	1480	BOTH KIDNEYS MOTTLED; ALL OTHER TISSUES APPEAR NORMAL
	1481	ALL TISSUES APPEAR NORMAL
	1482	ALL TISSUES APPEAR NORMAL
	1483	ALL TISSUES APPEAR NORMAL
	1484	ALL TISSUES APPEAR NORMAL
	1485	ALL TISSUES APPEAR NORMAL
	1486	ALL TISSUES APPEAR NORMAL

APPENDIX B

SPONSOR: Environmental Protection Department,
US Army Medical Bioengineering Research
and Development Laboratory

MATERIAL: Diisopropylmethylphosphonate (DIMP)

SUBJECT: FINAL REPORT
Analysis of Diet Formulations
LBI Project No. 10734

1. OBJECTIVE

The purpose of the study was to analyze DIMP in animal chow with regard to stability and formulation content in diet.

2. MATERIAL AND EXPERIMENTAL DESIGN

Analysis of the dosed feed was performed by the following chromatographic method:

Scope

This method describes the analytical procedure for the determination of DIMP in dosed feed used by Litton Bionetics, Inc. (LBI) from July, 1977 to September, 1978.

Principle

A five gram subsample is extracted with 15 ml of acetone by shaking for 10 minutes in an automatic shaker. The extract is clarified by centrifugation for 10 minutes at 1350 rpm and decanted into a separate tube. A second extraction is performed with 10 ml of acetone in the same manner. The two extracts are combined and centrifuged again prior to analysis by gas-liquid chromatography.

The amount of DIMP found is calculated by reference to calibration curves prepared by analysis of standard solutions of DIMP in acetone.

Equipment and Suppliers:

50 ml graduated conical Falcon tubes with positive seal caps (available from Becton, Dickinson, and Company, Oxnord, CA 93030), stock number H8292-209811

Short-stem glass funnels (corning 6180).

15 ml graduated glass centrifuge tubes with ground glass stoppers.

Centrifuge.

Volumetric glassware - 1, 4, and 10 ml pipettes; 50 and 100 ml flasks.

2. MATERIALS AND EXPERIMENTAL DESIGN (Continued)

Graduated cylinders - 25 ml capacity.

Mechanical shaker.

Analytical laboratory balance (accurate to 0.01 mg).

Top-loading laboratory balance (accurate to 0.01 g).

Gas-liquid chromatograph - Varian 2100, equipped with 1.8 m x 2 mm I.D. glass column packed with 10% FFAP on 80/100 mesh Supelcoport, flame ionization detectors.

Acetone (Burdick & Jackson).

Preparation of Standard:

Prepare a stock standard solution of DIMP by dissolving 75 mg of DIMP in 50 ml of acetone.

Take a 10 ml aliquot and dilute to 100 ml with acetone in a volumetric flask. This solution has a concentration of 0.15 mg/ml.

Prepare a standard curve by injecting 1, 2 and 3 μ l of the standard solution at the following parameters:

Column temperature:	120°C
Injector temperature:	250°C
FID temperature:	275°C
Chart:	6 minutes/inch
Carrier gas flow:	40 cc/min nitrogen
Attn:	16 x 10 ⁻¹¹

Procedure:

Weigh a 5 g sample of the dosed feed to the nearest 0.01 g in a Falcon tube.

Extract the sample with 15 ml of acetone by mixing for 10 min in a mechanical shaker, followed by centrifugation at 1300 rpm for 10 min.

Decant the supernatant into a Falcon tube and tightly seal to prevent solvent loss.

Repeat the extraction one additional time with 10 ml of acetone. Combine the extracts in the Falcon tube. Stopper tightly and mix well. Allow the sample to settle for 1 min, then centrifuge for 10 min at 1350 rpm.

Dilute the high-dose level (3000 ppm) in a 15 ml graduated centrifuge tube by adding a 1 ml aliquot to 4 ml of acetone.

2. MATERIAL AND EXPERIMENTAL DESIGN (Continued)

Repeat above procedure using undosed animal feed of the same lot used for the preparation of the dosed feed. This extract will be used as a negative control to assure that there are no interfering peaks contributed by the feed itself.

Repeat above procedure using undosed animal feed of the same lot which has been spiked in the laboratory with DIMP at corresponding dose levels.

Quantitate the amount of DIMP in solution by comparing to calibration curve prepared above.

Calculations:

Calculate the ppm of DIMP in the dosed feed or spiked (recovery) sample as follows:

To determine mg of sample injected:

$$\frac{5 \text{ g feed}}{25 \text{ ml acetone}} = \frac{200 \text{ mg feed}}{1.0 \text{ ml acetone}}$$

$$\frac{200 \text{ mg feed}}{1.0 \text{ ml acetone}} \times \text{Dilution Factor} = \frac{(x) \text{ mg feed}}{1.0 \text{ ml acetone}}$$

$$\begin{aligned} \text{Dilution Factor} &= 1 \text{ for 300 ppm level} \\ &= 0.2 \text{ for 3000 ppm level} \end{aligned}$$

$$\frac{(x) \text{ mg feed}}{1.0 \text{ ml acetone}} \times \frac{\mu\text{l sample}}{1000} = \text{mg of feed injected}$$

Calculate the intercept and slope from standard curve, as determined by linear regression correlation.

$$\frac{\text{Peak Response (peak height)} - \text{intercept}}{\text{slope}} = \text{ng of DIMP in injection}$$

To determine ppm:

$$\frac{\text{ng of DIMP}}{\text{mg of feed injected}} = \text{ppm}$$

Determine method recovery from spiked samples as follows:

$$\text{percent recovery} = \frac{\text{ppm found} \times 100}{\text{ppm added}}$$

Correct the result of the dosed feed sample for method recovery for its corresponding spiked sample.

$$\text{corrected ppm} = \frac{\text{sample ppm} \times 100}{\text{percent recovery}}$$

3. MATERIAL AND EXPERIMENTAL DESIGN

(Method Modification of 17 September 1978)

Scope

This method describes the analytical procedure used for the determination of DIMP in dosed feed used by Litton Bionetics, Inc. (LBI) from 17 September 1978 to the termination of the study.

Principle

A five gram subsample is extracted with 15 ml of acetone by shaking for 10 min in an automatic shaker. The extract is clarified by centrifugation for 10 min at 1350 rpm and decanted into a separate tube. A second extraction is performed with 10 ml of acetone in the same manner. The two extracts are combined and centrifuged again prior to analysis by gas-liquid chromatography.

An aliquot of the extract is diluted with a standard solution of trimethyl phosphate ($\text{CH}_3\text{O})_3\text{P}(\text{O})$. The trimethyl phosphate serves as an internal standard.

Analysis is performed by gas-liquid chromatography using a Hewlett-Packard 5840A with a 7672 Auto Liquid Sampler. Quantitation is by the automatic internal standard method.

Equipment and Supplies:

The basic laboratory equipment previously listed (see Section 2) is amended to include:

Gas-liquid chromatograph - Hewlett Packard 5840A with 7672 Auto Liquid Sampler equipped with 1.8 m x 2 mm I.D. glass column packed with 10% FFAP on 80/100 mesh supelcoport flame ionization detector.

Trimethyl phosphate (Aldrich 13,219-5)

Septa vial (Wheaton Industries 07081)

Preparation of Standards:

Prepare a stock standard solution of DIMP by dissolving 75 mg of DIMP in 50 ml of acetone.

Take a 4 ml aliquot and dilute to a final volume of 100 ml in a volumetric flask. This solution has a concentration of 0.06 mg/ml.

Prepare a stock standard solution of Trimethyl phosphate by dissolving 75 mg of trimethyl phosphate in 50 ml of acetone.

Take a 50 ml aliquot and dilute to a final volume of 100 ml in a volumetric flask. This solution has a concentration of 0.75 mg/ml.

3. MATERIAL AND EXPERIMENTAL DESIGN (Continued)

To prepare a working standard, take a 4 ml aliquot of the 0.06 mg/ml DIMP solution and combine with 4 ml of the 0.75 mg/ml trimethyl phosphate solution. Inject 3 μ l of the solution 4 times using the internal standard method (see Calculations, section 3 for description).

Procedure:

Weigh a 5 g sample of the dosed feed to the nearest 0.01 g into a Falcon tube.

Extract the sample with 15 ml of acetone by mixing for 10 min in a mechanical shaker followed by centrifugation at 1300 rpm for 10 min.

Decant the supernatant into a second Falcon tube and tightly seal to prevent solvent loss.

Repeat the extraction one additional time with 10 ml of acetone. Combine the extracts in the second Falcon tube. Stopper tightly and mix well. Allow the sample to settle for one minute, then centrifuge for 10 min at 1350 rpm.

Dilute the high-dose level (3000 ppm) in a 15 ml graduated centrifuge tube by taking a 1 ml aliquot with 9 ml of acetone.

Dilute 4 ml of the extract with 4 ml of trimethyl phosphate. The extract is now ready for analysis by gas chromatography.

Repeat above procedure using undosed animal feed of the same lot used for the preparation of the dosed feed. This extract will be used as a negative control and will assure that there are no interfering peaks contributed by the feed itself.

Repeat above procedure using undosed animal feed of the same lot which has been spiked in the laboratory with DIMP at corresponding dose levels.

Inject 3 μ l using the 7672 Auto Liquid Sampler, and calculate using internal standard method.

Calculations:

Calculate the ppm of DIMP in the dosed feed or spiked (recovery) sample as follows:

Determine mg of sample injected.

$$\frac{5 \text{ g feed}}{25 \text{ ml acetone}} = \frac{200 \text{ mg feed}}{1.0 \text{ ml acetone}}$$

$$\frac{200 \text{ mg feed}}{1.0 \text{ ml acetone}} \times \text{Dilution Factor} = \frac{(x) \text{ mg feed}}{1.0 \text{ ml acetone}}$$

$$\begin{aligned} \text{Dilution Factor} &= 1 \text{ for 300 ppm level} \\ &= 0.1 \text{ for 3000 ppm level} \end{aligned}$$

3. MATERIAL AND EXPERIMENTAL DESIGN (Continued)

Instrumental Dilution Factor = $1/(x)$ mg feed injected
= 2.5 for 300 ppm level
= 25 for 3000 ppm level

The 5840A Gas Chromatograph is a microprocessor-based instrument which is capable of automatically calculating analytical results by internal programming. A standard is established by making four equal injections of the DIMP standard solution and averaging the results. The nanograms of DIMP injected using the STND corresponds to the nanograms of DIMP in the samples (achieved by dilution if necessary). The actual analytical sequence used is:

$$\text{Concentration of DIMP (ppm)} = \frac{\text{Area 1} \times \text{Response 1}}{\text{Area 2} \times \text{Response 2}} \times \frac{\text{Amount I.S.}}{\text{I.S.}} \times \text{IDF}$$

Area 1 = Area of DIMP peak
Response 1 = Response of DIMP peak
Area 2 = Area of trimethyl phosphate peak
Response 2 = Response of trimethyl phosphate
Amount I.S. = Amount of trimethyl phosphate present in the injection in ng.
I.D.F. = Instrumental dilution factor (corrects to ppm)

Determine method recovery from spiked samples as follows:

$$\text{Percent recovery} = \frac{\text{ppm found} \times 100}{\text{ppm added}}$$

Correct the result of the dosed feed sample for method recovery of its corresponding spiked sample.

$$\text{corrected ppm} = \frac{\text{sample ppm} \times 100}{\text{Percent recovery}}$$

4. RESULTS

Stability Analysis

Samples were analyzed the day the mix was received by the analytical laboratory. This corresponded to Day 5 of the stability study. Two aliquots of feed were removed from each diet level of samples 0417, 0418, and 0419 and stored at ambient conditions. One aliquot was stored in a closed container, while the other was stored in an open container.

The two aliquots were analyzed five days later (Day 10) by the standard method. Results of the analysis are indicated in Table 1.

In the open containers, the concentration dropped 18.3% for the 300 ppm level and 31.3% for the 3000 ppm level.

In the closed container, the concentration remained the same for the 300 ppm level and dropped 9.3% for the 3000 ppm level.

5. DISCUSSION

The results of the stability study indicated that DIMP was not lost from the feed mixes stored in closed containers, whereas considerable loss occurred from open containers. The loss, therefore, appears to result from volatility of the compound.


The concentration of test compound found in feed mixes prepared for the rat reproduction study (Table 2) varied significantly at each dose level. In general, the amount of DIMP found in the 300 ppm diets were 83% of the intended dosage and at the higher dose level, the concentrations found averaged 88% of the theoretical dosage.

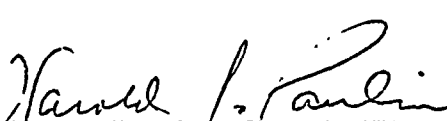
The levels of DIMP found in the diet mixes in Table 3 also showed significant variation. The amount of DIMP in the low level diet averaged about 77% of the intended dose level. However, the concentrations found in the two higher levels were usually within $\pm 10\%$ of the theoretical dosage.

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TABLE I-E-40

COMPARISON OF EXTRACTION PROCEDURES FOR DIMT

INITIAL METHOD					CURRENT METHOD				
<p>Perform Gas - Chromatographic Analysis</p>					<p>Add 5 Grams of Feed Spike Control Feed with Stock DIMP Solution for Method Recovery</p>				
Spike 1	Spike 2	Control	300 PPM Sample	3000 PPM Sample	Spike 1	Spike 2	Control	300 PPM Sample	3000 PPM Sample
1 ml	10 ml				1 ml	10 ml			
14 ml	5 ml	15 ml	15 ml	15 ml	14 ml	5 ml	15 ml	15 ml	15 ml
<p>Centrifuge and Decant Combine Extracts - Final Volume 25 ml</p>					<p>Centrifuge and Decant Supernatant Second Extraction with 10 ml Acetone</p>				
10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	10 ml
25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml	25 ml
<p>Perform Gas - Chromatographic Analysis</p>					<p>Dilute to Make all Sample Equal Concentration</p>				
					1:10				
60PPM	60PPM	60PPM	60PPM	60PPM	60PPM	60PPM	60PPM	60PPM	60PPM
<p>All Sample Equivalent in concentration</p>					<p>All Sample Equivalent in concentration</p>				
<p>Gas - Chromatographic Analysis Standard 3 µl Injection</p>					<p>Gas - Chromatographic Analysis Standard 3 µl Injection</p>				

TABLE I-E-41

LITTON BIONETICS, INC.
PROJECT 10715

TABLE 1

STABILITY OF DIMP IN DIET
MIXED 07/14/77

<u>SAMPLE NUMBER</u>	<u>ANALYSIS DATE</u>	<u>DAY</u>	<u>DOSAGE PPM</u>	<u>ANALYSIS VALUE (PPM)^a</u>
0417	07/19/77	5	0	0
0418	07/19/77	5	300	252
0419	07/19/77	5	3000	2882
Closed Container				
0417	07/27/77	10	0	0
0418	07/27/77	10	300	254
0419	07/27/77	10	3000	2614
Open Container				
0417	07/27/77	10	0	0
0418	07/27/77	10	300	206
0419	07/27/77	10	3000	1980

^aAll values have been corrected for respective method recovery, run simultaneously with analyses.

TABLE I-E-42

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY WEEK</u>	<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>PPM LEVEL</u>	<u>ANALYSIS VALUE (ppm)^a</u>
1	04/07/77	07/18/77	b	300	194
			b	3000	2581
2	04/14/77	07/18/77	b	300	209
			b	3000	2452
3	04/21/77	07/18/77	b	300	178
			b	3000	2339
4	04/28/77	07/18/77	b	300	218
			b	3000	2178
5	05/05/77	07/18/77	b	300	242
			b	3000	3226
6	05/12/77	07/18/77	b	300	2581 ^c
			b	3000	306 ^c
10	06/09/77	07/19/77	0336	0	0
			0337	300	240
			0338	3000	2282
11	06/16/77	07/13/77	0366	0	0
			0367	300	267
			0368	3000	2786
15	07/14/77	07/19/77	0417	0	0
			0418	300	252
			0419	3000	2882
16	07/21/77	07/27/77	0430	0	0
			0431	300	246
			0432	3000	3010
17	07/28/77	08/23/77	0459	0	0
			0460	300	185
			0461	3000	2554

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

^bSamples received in quart jars with no I.D. number.

^cPossible mixup of the jar label indicated.

TABLE I-E-42 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2 (CONTINUED)

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY. WEEK</u>	<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>PPM LEVEL</u>	<u>ANALYSIS VALUE (ppm)^a</u>
19	08/11/77	08/23/77	0499	300	199
			0500	3000	2316
20	08/18/77	09/07/77	b	300	182
				3000	2348
21	08/25/77	09/07/77	0526	0	0
			0527	300	244
			0528	3000	2386
24	09/15/77	09/27/77	0616	0	0
			0617	300	199
			0618	3000	2392
27	10/06/77	10/25/77	0689	300	316
			0688	3000	2297
28	10/14/77	10/25/77	0748	300	244
			0747	3000	2511
29	10/20/77	10/25/77	0796	0	
			0797	300	333
			0798	3000	2547
30	10/27/77	11/03/77	0855	300	189
			0857	3000	2136
31	11/03/77	11/11/77	0922	300	204
			0923	3000	2294
36	12/09/77	12/15/77	1095	0	0
			1096	300	0 ^c
			1097	3000	2516
			1128	300	253 ^d
		12/16/77			

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

^bSamples received in quart jars with no I.D. number.

^cAnalysis repeated to confirm results, sample resubmitted and analyzed immediately.

^dRepeat of 1096.

TABLE I-E-42 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2 (CONTINUED)

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY . WEEK</u>	<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>PPM LEVEL</u>	<u>ANALYSIS VALUE (ppm)^a</u>
38	12/22/77	12/28/77	1161	0	0
			1162	300	206
			1163	3000	3024
39	12/28/77	01/05/78	1188	0	0
			1189	300	234
			1190	3000	2749
40	01/04/78	01/10/78	1229	0	0
			1230	300	184
			1231	3000	2285
41	01/11/78	01/13/78	1262	0	0
			1263	300	231
			1264	3000	2679
42	01/19/78	01/22/78	1293	0	0
			1294	300	247
			1295	3000	2700
42	01/19/78	01/25/78	1293	0	0 ^b
			1294	300	239
			1295	3000	2335
43	01/24/78	02/03/78	1319	0	0
			1320	300	271
			1321	3000	2860
44	02/01/78	02/03/78	FC0047K78	0	0
			FC0048K78	300	271
			FC0049K78	3000	3180
45	02/08/78	02/13/78	FC0152K78	0	0
			FC0153K78	300	255
			FC0154K78	3000	2755
46	02/15/78	02/19/78	FC0212K78	0	0
			FC0213K78	300	256
			FC0214K78	3000	2836

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

^bRepeat analysis to confirm original results.

TABLE I-E-42 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2 (CONTINUED)

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY, WEEK</u>	<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>PPM LEVEL</u>	<u>ANALYSIS VALUE (ppm)^a</u>
47	02/22/78	02/23/78	FC0306K78	0	0
			FC0307K78	300	300
			FC0308K78	3000	2912
48	03/02/78	03/08/78	FC0437K78	0	0
			FC0438K78	300	240
			FC0439K78	3000	2631
49	03/08/78	03/13/78	FC0517K78	0	0
			FC0518K78	300	301
			FC0519K78	3000	2517
50	03/15/78	03/20/78	FC0653K78	0	0
			FC0654K78	300	239
			FC0655K78	3000	2732
51	03/21/78	03/23/78	FC0727K78	0	0
			FC0728K78	300	249
			FC0729K78	3000	2867
52	03/29/78	04/02/78	FC0819K78	0	0
			FC0820K78	300	231
			FC0821K78	3000	2628
53	04/05/78	04/06/78	FC0901K78	0	0
			FC0902K78	300	263
			FC0903K78	3000	2452
54	04/12/78	04/13/78	FC0972K78	0	0
			FC0973K78	300	278
			FC0974K78	3000	2759
55	04/19/78	04/19/78	FC1017K78	0	0
			FC1018K78	300	269
			FC1019K78	3000	2822
56	04/26/78	04/27/78	FC1087K78	0	0
			FC1088K78	300	299
			FC1089K78	3000	3022

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

TABLE I-E-42 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2 (CONTINUED)

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY.</u> <u>WEEK</u>	<u>MIX</u> <u>DATE</u>	<u>ANALYSIS</u> <u>DATE</u>	<u>SAMPLE</u> <u>NUMBER</u>	<u>PPM</u> <u>LEVEL</u>	<u>ANALYSIS</u> <u>VALUE (ppm)^a</u>
57	05/03/78	05/04/78	FC1143K78	0	0
			FC1144K78	300	274
			FC1145K78	3000	2821
58	05/10/78	05/13/78	FC1192K78	0	0
			FC1193K78	300	249
			FC1194K78	3000	2276
59	05/17/78	05/21/78	FC1244K78	0	0
			FC1245K78	300	275
			FC1246K78	3000	2907
60	05/24/78	05/31/78	FC1302K78	0	0
			FC1303K78	300	263
			FC1304K78	3000	2619
61	05/31/78	06/02/78	FC1336K78	0	0
			FC1337K78	300	257
			FC1338K78	3000	3003
62	06/07/78	06/11/78	FC1402K78	0	0
			FC1403K78	300	316
			FC1404K78	3000	3146
63	06/14/78	06/14/78	FC1467K78	0	0
			FC1468K78	300	286
			FC1469K78	3000	3133
64	06/21/78	06/27/78	FC1570K78	0	0
			FC1571K78	300	234
			FC1572K78	3000	2608
65	06/28/78	07/08/78	R0121K78	0	0
			R0122K78	300	227
			R0123K78	3000	2423
66	07/05/78	07/08/78	R0143K78	0	0
			R0144K78	300	269
			R0145K78	3000	3033

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

TABLE I-E-42 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2 (CONTINUED)

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY.</u> <u>WEEK</u>	<u>MIX</u> <u>DATE</u>	<u>ANALYSIS</u> <u>DATE</u>	<u>SAMPLE</u> <u>NUMBER</u>	<u>PPM</u> <u>LEVEL</u>	<u>ANALYSIS</u> <u>VALUE (ppm)^a</u>
67	07/13/78	07/16/78	R0172K78	0	0
			R0173K78	300	269
			R0174K78	3000	2992
68	07/20/78	07/25/78	b	0	0
				300	194
				3000	2101
69	07/26/78	07/28/78	R0220K78	0	0
			R0221K78	300	198
			R0222K78	3000	2575
70	08/03/78	08/07/78	R0267K78	0	0
			R0268K78	300	253
			R0269K78	3000	2971
71	08/10/78	08/14/78	R0327K78	0	0
			R0328K78	300	293
			R0329K78	3000	2851
72	08/17/78	08/20/78	R0376K78	0	0
			R0377K78	300	270
			R0378K78	3000	2748
73	08/24/78	08/27/78	R0489K78	0	0
			R0490K78	300	274
			R0491K78	3000	2858
74	08/31/78	09/02/78	R0525K78	0	0
			R0526K78	300	257
			R0527K78	3000	2870
76	09/14/78	09/17/78	R0595K78	0	0
			R0596K78	300	267
			R0597K78	3000	2746

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

^bNo sample number indicated.

TABLE I-E-42 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734

TABLE 2 (CONTINUED)

WEEKLY DIMP FEED ANALYSIS - RAT REPRODUCTION STUDY

<u>STUDY. WEEK</u>	<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>PPM LEVEL</u>	<u>ANALYSIS VALUE (ppm)^a</u>
77	09/20/78	09/30/78	R0617K78	0	0
			R0618K78	300	279
			R0619K78	3000	1705
78	09/26/78	09/30/78	R0662K78	0	0
			R0663K78	300	277
			R0664K78	3000	2745
79	10/03/78	10/17/78	R0694K78	0	0
			R0695K78	300	300
			R0696K78	3000	2799
81	10/17/78	10/24/78	R0754K78	0	0
			R0755K78	300	trace
			R0756K78	3000	2760
82	10/25/78	10/26/78	R0782K78	0	0
			R0783K78	300	293
			R0784K78	3000	3040
83	11/01/78	11/05/78	R0817K78	0	0
			R0818K78	300	241
			R0819K78	3000	2513
84	11/07/78	11/10/78	R0834K78	0	0
			R0835K78	300	250
			R0836K78	3000	2698
85	11/14/78	11/16/78	R0874K78	0	0
			R0875K78	300	242
			R0876K78	3000	2632
86	11/21/78	11/25/78	R0897K78	0	0
			R0898K78	300	296
			R0899K78	3000	3106

^aAll values have been corrected for respective method recovery run simultaneously with analysis.

PART I - SECTION F
DEMYELINATION PARALYSIS IN CHICKENS

DIMP

LBI PROJECT NO. 2566

SUMMARY

Treatment of atropinized White Leghorn hens with DIMP once at 1500 mg/kg or twice at 1000 or 500 mg/kg did not produce evidence of nerve fiber degeneration after 21 days (animals treated once) or 46 days (animals treated twice) when the sciatic nerve was examined microscopically. Evidence of unsteady gait was easily distinguished clinically from signs exhibited by the positive control animals and was judged to be unrelated to nerve fiber degeneration.

The positive control animals, treated with TOCP once at 500 mg/kg, developed classical progressive clinical signs of nerve fiber degeneration beginning 12 to 14 days after treatment. Most of the animals showed microscopic evidence of nerve fiber degeneration.

1. OBJECTIVE

The objective of this study was to determine the potential of the test material to cause demyelination of the sciatic nerve in hens after one or two single oral treatments.

2. MATERIAL

Refer to Part I - Section A.

3. EXPERIMENTAL DESIGN

Young adult Leghorn hens (weighing 1.1 to 1.8 kg and 18 to 20 weeks of age at the time of treatment, February 10, 1978) were obtained from Bowman's Hatchery, Westminster, Maryland, and acclimated to laboratory conditions for 13 days. The hens were singly housed in stainless steel rabbit cages in a temperature-controlled room with artificial illumination automatically controlled to provide a 12-hour light cycle. Growena chicken meal and water were provided ad libitum, except the night before treatment when food was removed from the cages. No other test chemicals were under concurrent investigation in this animal room. This study was performed in the Falls Church facility of the Toxicology Department.

3. EXPERIMENTAL DESIGN (Continued)

The dose levels of DIMP administered to the test animals were selected based on data generated during the acute oral toxicity study in chickens, dated September 8, 1977. Treatment mixtures of DIMP and TOCP were prepared in corn oil. Atropine sulfate was prepared using deionized water and was made to a concentration of 54 mg/ml. The specific gravities for DIMP and TOCP were 1.0 and 1.16, respectively. Fresh treatment mixtures were prepared for each use.

The study design has been summarized in Text Table A. Each animal received a measured volume of atropine sulfate solution (54 mg/ml) prior to administration of the test compounds or vehicle control so that a dose of 15 mg/kg was received. Typically, 15 minutes elapsed between the time of atropine administration and treatment with the test compound.

The animals were treated and observed for 21 days. At Day 22, surviving animals from the high dose group, the positive control group and half of the negative control animals were killed. The remaining animals were redosed with atropine sulfate and their respective treatment mixtures, and observed for an additional 24 days. This second treatment was performed on the low and intermediate dose level DIMP animals because there were no indications of neural toxicity after 21 days. The hens in the high DIMP dose level were not redosed since significant mortality resulted after the initial treatment.

The animals were observed twice daily for mortality and general appearance. Particular attention was given to observing the gait of the hens, since demyelination of the sciatic nerve and its concomitant disruption of normal walking and standing was the parameter under investigation. All animals were weighed prior to treatment.

Animals dying within 96 hours of treatment were necropsied to ensure proper dosing technique only. Animals dying after 96 hours of treatment, as well as all animals terminated, were subjected to a special truncated necropsy procedure. During this necropsy, the left leg sciatic nerves were dissected and preserved in 10% formalin. The five nerve junctions with the spinal cord were left intact and the entire nerve (encompassing sciatic, tibial and plantar nerves) leading to the foot was removed. No other tissues were observed for gross lesions or preserved.

TABLE I-F-43

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE A

STUDY DESIGN

<u>GROUP NUMBER</u>	<u>GROUP DESIGNATION</u>	<u>COMPOUND</u>	<u>DOSE LEVEL (MG/KG)</u>	<u>TEST MATERIAL CONCENTRATION (MG/ML)</u>	<u>ANIMAL NUMBERS</u>
1	Vehicle Control	Corn Oil	0	0	16-35
2	Positive Control	TOCP	500	250	36-55
3	Treatment	DIMP	500	250	56-75
4	Treatment	DIMP	1000	500	76-95
5	Treatment	DIMP	1500	750	96-115

Date of first dose: February 10, 1978, all groups.

Date of second dose: March 3, 1978, groups 1*, 3, 4.

Date of first terminal kill: March 3, 1978, groups 1*, 2, 5.

Date of second terminal kill: March 27, 1978, groups 1, 3, 4.

*Nine animals only.

4. RESULTS

Mortality data has been summarized in Appendix Table 1. Two control hens were judged moribund and killed on Days 18 and 41 of study. There were no deaths among the positive control animals. Seven hens in the high dose and five hens in the medium dose were killed shortly after first treatment with DIMP. These deaths were clearly related to treatment with the test compound. One hen in the medium dose and two hens in the low dose were found dead after the second dosing. Additionally, three medium dose hens were judged moribund and killed.

Clinical signs have been summarized in Appendix Table 2. Predictably, animals treated with TOCP developed unsteady gait on Days 12 to 14 (19 hens of the 20 treated were affected). This condition progressed as described below until termination at Day 21, in 17 animals. In two instances the animals finally could neither walk nor stand.

The animals treated with DIMP showed toxic signs, the severity of which was related to the dose administered. Within minutes of treatment, particularly in the high dose, the hens became very quiet and soon were prostrate. While these animals gave the appearance of being very close to death, most recovered. Many of the surviving hens had an unsteady gait on Days 1 to 3 after treatment. This unsteadiness was judged to be related to the test compound.

Among the animals treated with 500 mg/kg of DIMP initially, all animals remained clinically normal throughout the 21 day observation period. When these animals were treated a second time with 500 mg/kg of the compound, five animals (Nos. 57, 58, 60, 65, 67) developed signs of unsteady gait or inability to walk. In four of these animals, the signs were judged unsimilar to those of the positive control. In assessing these signs, they were compared to the characteristics shown by the positive control animals. These characteristics were:

- a. The regular onset of "unsteady gait" 12 to 14 days after initial treatment in those animals affected (95% of treated). This regularity was seen both temporally and in severity. Eleven animals were judged, initially, to show "slightly unsteady gait". All of these then progressed to "unsteady gait" and seven also were judged by Day 19 to 20 to show "very unsteady gait". Eight animals were judged, initially, to show "unsteady gait". Five of these progressed to "very unsteady gait" by Day 19 to 20 (with one hen, No. 38, showing "very unsteady gait" as early as Day 16) and two animals (Nos. 38 and 54) progressed to "unable to stand" by Day 19 to 20. Thus, 16 animals showed progressive signs, three showed steady signs and one remained normal.

4. RESULTS (Continued)

- b. The gradual progressiveness of the "unsteady gait" once this sign was evidenced.

In general, the "slight" to "unsteady" to "very unsteady" changes in clinical condition were noted on Days 13, 14 and 19. There were no instances of dramatic rapid deterioration of the animals.

- c. The failure of any affected animals to improve, clinically, in the time frame of this study.

Among the animals treated with 1000 mg/kg of DIMP, initially, 13 of the surviving 16 animals were normal (neglecting the early signs of toxicity after treatment) for the entire initial observation period. One animal (No. 82) was normal for 20 days, but deteriorated rapidly and was judged moribund on Day 21. Two animals (Nos. 93 and 95) both showed signs relating to unsteady gait, but returned to normal by the end of the initial observation period. Thus, using the criteria above, none of the animals treated once with 1000 mg/kg DIMP developed clinical signs similar to those shown by the positive control TOCP animals.

Among the 15 animals treated a second time with 1000 mg/kg DIMP, one animal died after treatment and eight remained normal (neglecting the initial signs of toxicity after treatment) throughout the additional 24 day observation period. The remaining six animals (three of which were judged moribund) showed signs that were not similar to the positive control animals and it was, again, judged that treatment with 1000 mg/kg DIMP was without similar effect as seen after TOCP treatment.

Among the surviving animals treated once with 1500 mg/kg DIMP, 11 of 12 hens were clinically normal for the 21 day observation period (neglecting the initial signs of toxicity after treatment). One animal (No. 100) was normal through Day 18, developed a slight unsteady gait for two days, but was again normal at Day 21. These signs were judged unsimilar to the positive control animals.

The summary of necropsy findings has been tabulated in Appendix Table 3. One animal (No. 80), treated twice with DIMP at 1000 mg/kg, showed slight muscle wasting on its left leg. There were no other remarkable findings relating to the musculature or sciatic nerve of the left leg of any of the animals.

4. RESULTS (Continued)

The pathology report, written by Herman Seibold, D.M.V. (LBI), has been appended. He examined the sciatic nerve of these hens using two sections of each specimen--one stained with hematoxylin and eosin, and the other stained with luxol-fast blue and counter stained with cresyl violet. It should be noted that the nerve specimens submitted for microscopic evaluation as dissected from the spinal cord to the foot included anatomically the sciatic, tibial and plantar nerves. Microscopic distinction of tibial and plantar segments, however, was not possible; thus, the results are discussed under the one category of sciatic nerve.

There was evidence of nerve fiber degeneration in 13 of the 19 hens that showed signs of unsteady gait after TOCP treatment. The severity of this degeneration averaged 1.8 on a scale that used 1 to indicate minimal, 2 to indicate slight and 3 to indicate moderate nerve fiber degeneration. The absence of microscopic lesions in six animals that showed clinical signs led Dr. Seibold to observe that the clinical manifestation of TOCP toxicity was more sensitive an indicator of TOCP induced nerve damage than routine microscopic evaluation.

There were lesions rated as "trace" in two hens treated with 1000 mg/kg and "slight" in one hen treated with 1500 mg/kg. One hen with trace amounts of degeneration was treated once with DIMP, was normal until Day 20 and became moribund on Day 21. The other hen in the 1000 mg/kg group was treated twice with DIMP. This hen was normal throughout the first observation period, normal for the first 20 days of the second observation period, unable to stand for the next three days but improved at Day 24, in that it was able to stand but unable to walk.

The one hen in the 1500 mg/kg group that had "slight" evidence of nerve lesions was normal (except for signs of initial acute toxicity) throughout its observation period.

There was no evidence of nerve fiber degeneration in any of the other hens receiving the vehicle alone, DIMP at 500 mg/kg, 1000 mg/kg or 1500 mg/kg. Dr. Seibold concluded that the trace lesions in the two hens treated with 1000 mg/kg DIMP were probably not unusual in light of similar lesions reported for normal hens and that the minimal lesion in the 1500 mg/kg hen was coincidental since this animal showed no clinical signs and he concluded that nerve fiber degeneration attributable to delayed neurotoxicity of DIMP was not apparent in these animals.

4. RESULTS (Continued)

These data indicate that DIMP does not induce nerve fiber degeneration in hens after either one treatment at 1500 mg/kg or two treatments at 1000 or 500 mg/kg. The high dose was the maximum that could be administered as judged by the mortality immediately following treatment. The delayed appearance of an unsteady gait observed in one animal (No. 60) after two treatments with 500 mg/kg had similar characteristics of the positive control animals, but is judged to be incidental and not related to nerve fiber degeneration.

The remaining clinical signs were different in one or more respects when compared to the positive control animals. Animals lacked substantive evidence of nerve fiber degeneration and it was judged that DIMP is without effect with regard to nerve fiber degeneration in hens.

Thirty days after transmittal of this report, original data from the Department of Toxicology will be transferred to the LBI Archivist, 5516 Nicholson Lane, Kensington, Maryland, for distribution to the proper repositories. A copy of this report was reviewed by the LBI Quality Assurance Unit.

5. CONCLUSION

Treatment of atropinized White Leghorn hens with DIMP once at 1500 mg/kg or twice at 1000 or 500 mg/kg did not produce evidence of nerve fiber degeneration after 21 days (animals treated once) or 46 days (animals treated twice) when the sciatic nerve was examined microscopically. Evidence of unsteady gait was easily distinguished clinically from signs exhibited by the positive control animals and was judged to be unrelated to nerve fiber degeneration.

The positive control animals, treated with TOCP once at 500 mg/kg, developed classical progressive clinical signs of nerve fiber degeneration beginning 12 to 14 days after treatment. Most of the animals showed microscopic evidence of nerve fiber degeneration.

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TABLE I-F-44

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE 1

MORTALITY

GROUP DESIGNATION	COMPOUND	DOSE LEVEL (MG/KG)	INITIAL DOSING			SECOND DOSING			TOTAL MORTALITY (DEAD/TREATED)
			MORTALITY BY DAY	1	2	3	4-21	CUMULATIVE MORTALITY (DEAD/TREATED)	
CONTROL	-	0	-	-	-	-	1	1/20	1/9
POSITIVE CONTROL	TOCP	500	-	-	-	-	-	0/20	0/20
TREATMENT	DIMP	500	-	-	-	-	-	0/20	2/20
TREATMENT	DIMP	1000	1	4	-	-	-	5/20	4/15
TREATMENT	DIMP	1500	3	4	-	-	-	7/20	-

TABLE I-F-45

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE 2

CLINICAL SIGNS

INITIAL DOSING

<u>DOSE GROUP (MG/KG)</u>	<u>ANIMAL NUMBER</u>	<u>DAY OF STUDY</u>	<u>SIGN</u>
CONTROL	17	1 ^a	QUIET; EYES SLIGHTLY CLOSED. DOWN IN CAGE AND UNABLE TO STAND; MORIBUND KILL.
	28	18	
TOCP 500	36	14-16	UNSTEADY GAIT.
		17	SLIGHTLY UNSTEADY GAIT.
		18-21	UNSTEADY GAIT.
	37	13-18	UNSTEADY GAIT.
		19-21	VERY UNSTEADY GAIT.
	38	14, 15	UNSTEADY GAIT.
		16-18	VERY UNSTEADY GAIT.
		19-21	SITTING, UNABLE TO STAND OR WALK.
	39	13	SLIGHTLY UNSTEADY GAIT.
		14-18	UNSTEADY GAIT.
		19-21	VERY UNSTEADY GAIT.
	40	13	SLIGHTLY UNSTEADY GAIT.
		14-19	UNSTEADY GAIT.
		20, 21	VERY UNSTEADY GAIT.
	41	14-21	UNSTEADY GAIT.
	42	14, 16, 17	SLIGHTLY UNSTEADY GAIT.
		15, 18-21	UNSTEADY GAIT.
	43	13-18	UNSTEADY GAIT.
		19-21	VERY UNSTEADY GAIT.
	44	13-18	UNSTEADY GAIT.
		19-21	VERY UNSTEADY GAIT.
	45	13	SLIGHTLY UNSTEADY GAIT.
		14-19	UNSTEADY GAIT.
		20, 21	VERY UNSTEADY GAIT.
	46	13	SLIGHTLY UNSTEADY GAIT.
		14-18	UNSTEADY GAIT.
		19-21	VERY UNSTEADY GAIT.
	47	13	SLIGHTLY UNSTEADY GAIT.
		14-18	UNSTEADY GAIT.
		19-21	VERY UNSTEADY GAIT.
	48	13-21	UNSTEADY GAIT.
	49	13	SLIGHTLY UNSTEADY GAIT.
		14-19	UNSTEADY GAIT.
		20, 21	VERY UNSTEADY GAIT.
	50	13	SLIGHTLY UNSTEADY GAIT.
		14-21	UNSTEADY GAIT.

^aDay 1 observations made one hour after treatment.

TABLE I-F-45 (Continued)

LITTON BIONETICS, INC.
PROJECT NO 20566

TABLE 2 (CONTINUED)

CLINICAL SIGNS

INITIAL DOSING

DOSE GROUP (MG/KG)	ANIMAL NUMBER	DAY OF STUDY	SIGN
TOCP 500	51	13-18	SLIGHTLY UNSTEADY GAIT.
		19-21	UNSTEADY GAIT.
	52	12, 13	SLIGHTLY UNSTEADY GAIT.
		14-19	UNSTEADY GAIT.
		20, 21	VERY UNSTEADY GAIT.
	54	12-18	UNSTEADY GAIT.
		19	VERY UNSTEADY GAIT.
		20, 21	SITTING, UNABLE TO STAND OR WALK.
DIMP 500	55	13-18	SLIGHTLY UNSTEADY GAIT.
		19-21	UNSTEADY GAIT.
	56	1	QUIET, UNABLE TO STAND.
	57	1	UNSTEADY GAIT.
	58	1	UNSTEADY GAIT.
	59	1	SLIGHTLY UNSTEADY GAIT.
	60	1	UNSTEADY GAIT.
	62	1	UNSTEADY GAIT.
	63	1	UNSTEADY GAIT.
	64	1	UNSTEADY GAIT.
	65	1	UNSTEADY GAIT.
	66	1	UNSTEADY GAIT.
	67	1	UNSTEADY GAIT, VERY QUIET.
	68	1	UNSTEADY GAIT.
	69	1	UNSTEADY GAIT.
	70	1	UNSTEADY GAIT.
	71	1	UNSTEADY GAIT.
	73	1	UNSTEADY GAIT.
	74	1	UNSTEADY GAIT.
	75	1	SLIGHTLY UNSTEADY GAIT.
DIMP 1000	76	1	PROSTRATE.
		2	UNSTEADY GAIT.
	77	1, 2	UNSTEADY GAIT.
		1	PROSTRATE.
	78	2	UNSTEADY GAIT.
		1	UNSTEADY GAIT.
	79	2	FOUND DEAD.
		1	PROSTRATE.
	80	2	VERY UNSTEADY GAIT.
		1	PROSTRATE.
	81	2	UNSTEADY GAIT.

TABLE I-F-45 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE 2 (CONTINUED)

CLINICAL SIGNS

INITIAL DOSING

<u>DOSE GROUP (MG/KG)</u>	<u>ANIMAL NUMBER</u>	<u>DAY OF STUDY</u>	<u>SIGN</u>
DIMP 1000	82	1	PROSTRATE.
		2	UNSTEADY GAIT.
		21	SITTING, UNABLE TO STAND OR WALK; MORIBUND KILL.
	83	1, 2	UNSTEADY GAIT.
	84	1, 2	UNSTEADY GAIT.
	85	1	PROSTRATE.
		2	FOUND DEAD.
	86	1	PROSTRATE.
		2	UNSTEADY GAIT.
	87	1	PROSTRATE.
		2	UNSTEADY GAIT.
		3	SLIGHT UNSTEADY GAIT.
	88	1	PROSTRATE.
		2	FOUND DEAD.
	89	1	PROSTRATE.
		2	UNSTEADY GAIT.
		3	SLIGHTLY UNSTEADY GAIT.
	90	1	PROSTRATE.
		2	VERY UNSTEADY GAIT.
		3	UNSTEADY GAIT.
	91	1	PROSTRATE.
		2	SLIGHTLY UNSTEADY GAIT.
	92	1	UNSTEADY GAIT.
		2	SLIGHTLY UNSTEADY GAIT.
	93	1	PROSTRATE.
		12-17	SLIGHTLY UNSTEADY GAIT.
	94	1	UNSTEADY GAIT.
		2	FOUND DEAD.
	95	1, 2	UNSTEADY GAIT.
		3	SLIGHTLY UNSTEADY GAIT.
		12-14	UNABLE TO STAND.
		15	VERY UNSTEADY GAIT.
	96	16, 17	UNSTEADY GAIT.
		18	SLIGHTLY UNSTEADY GAIT.
		1	FOUND DEAD.
DIMP 1500	97	1	UNSTEADY GAIT.
		2	VERY UNSTEADY GAIT.
		3	SLIGHTLY UNSTEADY GAIT.
	98	1	PROSTRATE.
		2	FOUND DEAD.

TABLE I-F-45 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE 2 (CONTINUED)

CLINICAL SIGNS

INITIAL DOSING

<u>DOSE GROUP (MG/KG)</u>	<u>ANIMAL NUMBER</u>	<u>DAY OF STUDY</u>	<u>SIGN</u>
DIMP 1500	99	1	PROSTRATE.
		2	FOUND DEAD.
	100	1	PROSTRATE.
		2	UNSTEADY GAIT.
	101	19, 20	SLIGHTLY UNSTEADY GAIT.
		1	PROSTRATE.
	102	2	UNSTEADY GAIT.
		1	FOUND DEAD.
	103	1	PROSTRATE.
		2	VERY UNSTEADY GAIT.
	104	3	UNSTEADY GAIT.
		1	FOUND DEAD.
	105	1	PROSTRATE.
		2	VERY UNSTEADY GAIT.
	106	3	SLIGHTLY UNSTEADY GAIT.
		1	PROSTRATE.
	107	2	VERY UNSTEADY GAIT.
		1	PROSTRATE.
	108	2, 3	VERY UNSTEADY GAIT.
		1	FOUND DEAD.
	109	1	PROSTRATE.
		2	UNSTEADY GAIT.
	110	1, 2	PROSTRATE.
		3	SLIGHTLY UNSTEADY GAIT.
	111	1, 2	PROSTRATE.
		3	SLIGHTLY UNSTEADY GAIT.
	112	1	PROSTRATE.
		2	FOUND DEAD.
	113	1, 2	UNSTEADY GAIT.
		3	SLIGHTLY UNSTEADY GAIT.
	114	1	PROSTRATE.
		2	FOUND DEAD.
	115	1, 2	UNSTEADY GAIT.

TABLE I-F-45 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE 2 (CONTINUED)

CLINICAL SIGNS

SECOND DOSING

<u>DOSE GROUP (MG/KG)</u>	<u>ANIMAL NUMBER</u>	<u>DAY OF STUDY</u>	<u>SIGN</u>
CONTROL	35	41	PROSTRATE, EYES CLOSED; MORIBUND KILL.
DIMP 500	56	22	SITTING, UNSTEADY GAIT WHEN TOUCHED.
	57	22	QUIET, SLIGHTLY UNSTEADY GAIT.
		43	SITTING, UNABLE TO STAND.
		44	STANDS, BUT WALKS WITH GREAT DIFFICULTY.
	58	45	WALKS, BUT UNSTEADY ON RIGHT LEG.
		22	UNSTEADY GAIT.
		34-45	UNSTEADY ON RIGHT LEG, JOINT ENLARGED.
	59	22	SLIGHTLY UNSTEADY GAIT.
	60	22	SITTING, SLIGHTLY UNSTEADY GAIT WHEN TOUCHED.
	61	41-45	SLIGHTLY UNSTEADY GAIT.
		22	STANDING, QUIET; UNSTEADY GAIT WHEN TOUCHED.
	62	22	SLIGHTLY UNSTEADY GAIT.
	63	22	SITTING, VERY UNSTEADY GAIT WHEN TOUCHED.
	64	22	STANDING WITH EYES CLOSED; UNSTEADY GAIT WHEN TOUCHED.
	65	22	SLIGHTLY UNSTEADY GAIT.
		35	PROSTRATE.
		36	PROSTRATE; NOT EATING OR DRINKING; MORIBUND KILL.
	66	22	STANDING, QUIET; UNSTEADY GAIT WHEN TOUCHED.
	67	22	SITTING, EYES CLOSED; SLIGHTLY REACTIVE WHEN TOUCHED.
	68	22	SLIGHTLY UNSTEADY GAIT.
	70	22	SLIGHTLY UNSTEADY GAIT.
	71	22	UNSTEADY GAIT.
	72	22	UNSTEADY GAIT.
	73	22	SITTING, EYES CLOSED; UNSTEADY GAIT WHEN TOUCHED.
	75	22	UNSTEADY GAIT.
DIMP 1000	76	22-24	PROSTRATE.
		25	SITTING, UNABLE TO STAND.
		26	PROSTRATE; COMB LIMP, EDGES PURPLE.
		27	FOUND DEAD.

TABLE I-F-45 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 20566

TABLE 2 (CONTINUED)

CLINICAL SIGNS

SECOND DOSING

<u>DOSE GROUP (MG/KG)</u>	<u>ANIMAL NUMBER</u>	<u>DAY OF STUDY</u>	<u>SIGN</u>
DIMP 1000	77	22	PROSTRATE.
	78	22	PROSTRATE.
	80	22	PROSTRATE.
		23	UNSTEADY GAIT.
		36-45	UNABLE TO STAND ON LEFT LEG.
	81	22	PROSTRATE.
		23	SLIGHTLY UNSTEADY GAIT.
		36, 37	PROSTRATE.
		38, 39	PROSTRATE; COMB LIMP, EDGES PURPLE.
		40	PROSTRATE; COMB LIMP, COLOR NORMAL.
	83	41	PROSTRATE; COMB LIMP; MORIBUND KILL.
		22	SITTING, SLIGHT MOVEMENT WHEN TOUCHED.
		36-41	SLIGHTLY UNSTEADY GAIT.
	84	22	PROSTRATE.
		23	SITTING/STANDING, UNSTEADY GAIT WHEN TOUCHED.
		42-44	PROSTRATE.
	86	45	STANDING, BUT UNABLE TO WALK.
		22	STANDING, QUIET; VERY UNSTEADY GAIT WHEN TOUCHED.
		36-40	PROSTRATE.
	87	41	PROSTRATE; MORIBUND KILL.
		22	PROSTRATE.
		23	SLIGHTLY UNSTEADY GAIT.
	89	22	PROSTRATE.
		23	SLIGHTLY UNSTEADY GAIT.
	90	22	SITTING, SLIGHT MOVEMENT WHEN TOUCHED.
		23	SITTING/STANDING, VERY UNSTEADY GAIT WHEN TOUCHED.
		22	PROSTRATE.
	91	22	STANDING, QUIET; UNSTEADY GAIT WHEN TOUCHED.
	92	22	PROSTRATE.
	93	22	SITTING, SLIGHT MOVEMENT WHEN TOUCHED.
	95	22	PROSTRATE.
		23	SLIGHTLY UNSTEADY GAIT.
		24-32	PROSTRATE.
		36	PROSTRATE; COMB LIMP.
		37	PROSTRATE; COMB LIMP, EDGES PURPLE;
		38	MORIBUND KILL.

SUBJECT: FINAL PATHOLOGY REPORT
Demyelination Paralysis in Chickens
LBI Project No. 20566

1. OBJECTIVE

The objective was to determine the status of selected nerve tissue specimens with regard to the presence and degree of demyelination. Comparable nerve tissue specimens from chickens treated with Tri-O-Tolyl phosphate were provided as control material to serve as a basis for the microscopic evaluation.

2. METHODS

Specimens of sciatic, tibial and plantar nerve were fixed in buffered 10% formalin solution, pH 7.0, dehydrated with ascending concentrations of ethyl alcohol, cleared with xylene and embedded in Paraplast R. Sciatic nerve of each chicken was embedded in one block, while the tibial and plantar nerve segments were embedded together in a second block.

Duplicate sections were prepared at 6 microns; one was stained with hematoxylin and eosin and the other with Luxol-fast blue and counterstained with cresyl violet.

3. DEFINITIONS

Demyelination is the historical term applied to nerve fiber changes associated with the delayed neurotoxicity of Tri-O-cresyl phosphate (Tri-O-Tolyl phosphate is a synonym). In a recent discussion of the pathology of delayed neurotoxicity due to organo-phosphates the following statement was made, "Basically the lesion is a "dying back" process or Wallerian degeneration in the axons although earlier work tended to consider it a demyelinating disease."¹ The term "die back" is used in another report² and agreement is expressed with the concept that Wallerian degeneration of the axon represents the primary morphologic effect of organo-phosphates with delayed neurotoxicity. However, this author used the designation "nerve fiber degeneration" in general discussion of the subject. The designation nerve fiber degeneration is therefore appropriate for the purpose of this report.

* Also known as TOCP.

4. RESULTS

A tabulation of nerve fiber degeneration in the different groups of chickens (vehicle controls, positive controls, low dose, medium dose and high dose) is given in Table 1.

None of the 20 chickens in the vehicle control group (of which nine were redosed) had microscopic evidence of nerve fiber degeneration. A total of 13 of the 20 chickens in the positive control group had microscopic evidence of nerve fiber degeneration with an average degree of 1.8 (1=minimal, 2=slight). This compares with 19 that had clinical signs and indicates that the microscopic examination was a less sensitive indicator of delayed neurotoxicity than the clinical signs under the conditions of this test.

None of the 20 chickens in the low dose group (of which all were redosed) had microscopic evidence of nerve fiber degeneration. Two of the 16 chickens in the middle dose group (of which 15 were redosed) had trace nerve fiber degeneration consisting of one short segment of a single nerve fiber showing degenerative changes.

One of the 12 chickens in the high dose group (of which none were redosed) had slight nerve fiber degeneration.

5. DISCUSSION

The nerve fiber degeneration in two of the middle dose (DIMP) chickens and in one of the high dose has debatable validity in the interpretation of the test. Trace lesions similar to those in two of the middle dose chickens have been reported to occur in odd nerve fibers in normal hens.¹ The high dose chicken with slight nerve fiber degeneration had not shown any clinical signs.

6. CONCLUSIONS

Nerve fiber degeneration that can be attributed to delayed neurotoxicity was not recognized in the various groups of chickens dosed with DIMP.

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¹Bradley, W.A.: The Pathology of Delayed Neurotoxicity Due to Organophosphates. In, Pesticide Induced Delayed Neurotoxicity, Proceedings of a Conference held in Washington, D.C., on February 19 and 20, 1976. US Department of Commerce, National Technical Information Service, PB-256 416. Prepared for National Institute of Environmental Health Sciences. pp 84-101, July 1, 1976.

²Cavanagh, J.B.: Peripheral Neuropathy Caused by Chemical Agents, CRC, Critical Reviews in Toxicology, 2:365-417, November, 1973.

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LEGEND TO TABLE OF MICROSCOPIC OBSERVATIONS

Column "A" = Animal number

Column "B" = Chicken disposition

TK = killed at termination of test.

MK = killed when moribund.

FD = found dead.

* = chicken did not survive for delayed neurotoxicity observation.

Column "C" = Nerve fiber degeneration

- = microscopic lesions not recognized.

1 = present in minimal degree.

2 = present in slight degree.

3 = present in moderate degree.

T = trace (one short segment of a single nerve fiber showing degenerative changes).

TABLE I-F-46

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TABLE 1

MICROSCOPIC OBSERVATIONS

Group #1 Vehicle Control			Group #2 Positive Control TOCP (500 mg/kg)			Group #3 Low Dose DIMP (500 mg/kg)			Group #4 Middle Dose DIMP (1000 mg/kg)			Group #5 High Dose DIMP (1500 mg/kg)		
A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
16	TK	-	36	TK	-	<u>Redosed Chickens</u>			82	MK	T	97	TK	-
17	TK	-	37	TK	2	56	TK	-	<u>Redosed Chickens</u>			100	TK	-
18	TK	-	38	TK	3	57	TK	-	<u>Redosed Chickens</u>			101	TK	-
19	TK	-	39	TK	3	58	TK	-	77	TK	-	103	TK	-
20	TK	-	40	TK	-	59	TK	-	78	TK	-	105	TK	-
21	TK	-	41	TK	1	60	TK	-	80	TK	-	106	TK	-
22	TK	-	42	TK	1	61	TK	-	83	TK	-	107	TK	-
23	TK	-	43	TK	1	62	TK	-	84	TK	T	109	TK	-
24	TK	-	44	TK	2	63	TK	-	87	TK	-	110	TK	2
25	TK	-	45	TK	-	64	TK	-	89	TK	-	111	TK	-
28	MK	-	46	TK	1	66	TK	-	90	TK	-	113	TK	-
<u>Redosed Chickens</u>			47	TK	-	68	TK	-	91	TK	-	115	TK	-
26	TK	-	48	TK	2	69	TK	-	92	TK	-	96	*	*
27	TK	-	49	TK	2	70	TK	-	93	TK	-	98	*	*
29	TK	-	50	TK	-	71	TK	-	81	MK	-	99	*	*
30	TK	-	51	TK	-	72	TK	-	86	MK	-	102	*	*
31	TK	-	52	TK	2	73	TK	-	95	MK	-	104	*	*
32	TK	-	53	TK	-	74	TK	-	76	FD	-	108	*	*
33	TK	-	54	TK	2	75	TK	-	79	*	-	112	*	*
34	TK	-	55	TK	1	65	MK	-	85	*	-	114	*	*
35	MK	-				67	MK	-	88	*	-			
									94	*	-			

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0016
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4660
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - one lost in processing; other no lesion recognized.

ANIMAL NUMBER: 0017
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4661
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0018
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4662
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0019
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4663
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0020
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4664
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0021
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PII NUMBER: 78/4665
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0022
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4666
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0023
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4667
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0024
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4668
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0025
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4669
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0026
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4670
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

MISSING TISSUES:
Sciatic nerve - lost in processing.
Tibial nerve - lost in processing.

ANIMAL NUMBER: 0027
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4671
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0028
DOSAGE: 0 mg/kg
DATE OF DEATH: 2/27/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4672
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
Animal sacrificed.

MICROSCOPIC FINDINGS:
No lesion recognized.

MISSING TISSUES:
Tibial nerve - one lost in processing.

ANIMAL NUMBER: 0029
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4673
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0030
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4674
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0031
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4675
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0032
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4676
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0033
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4677
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0034
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4678
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0035
DOSAGE: 0 mg/kg
DATE OF DEATH: 3/22/78
DEATH: Moribund kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4679
GROUP NUMBER: 1
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0036
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 76/4680
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0037
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4681
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - slight nerve fiber degeneration.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0038
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4682
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Sciatic nerve - minimal nerve fiber degeneration.
Tibial nerve, Plantar nerve - moderate nerve fiber degeneration.

ANIMAL NUMBER: 0039
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4683
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - moderate nerve fiber degeneration.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0040
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4684
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0041
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4685
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - minimal nerve fiber degeneration.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0042
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4686
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - minimal nerve fiber degeneration.

ANIMAL NUMBER: 0043
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4687
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - minimal nerve fiber degeneration.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0044
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4688
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - one lost in processing, the other
slight nerve fiber degeneration in tissue.

ANIMAL NUMBER: 0045
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4689
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0046
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4690
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - minimal nerve fiber degeneration.

ANIMAL NUMBER: 0047
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4691
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0048
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4692
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - slight nerve fiber degeneration.

ANIMAL NUMBER: 0049
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4693
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - slight nerve fiber degeneration.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0050
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4694
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0051
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4695
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0052
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4696
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - slight nerve fiber degeneration.

ANIMAL NUMBER: 0053
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4697
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - one lost in processing; the other
no lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0054
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4698
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - slight nerve fiber degeneration.

ANIMAL NUMBER: 0055
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4699
GROUP NUMBER: 2
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - minimal nerve fiber degeneration.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0056
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4700
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0057
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4701
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0058
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4702
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0059
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4703
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0060
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4704
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0061
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4705
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

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INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0062
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4706
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0063
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4707
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0064
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4708
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

MISSING TISSUES:
Tibial nerve, Plantar nerve - both tissues lost in processing.

ANIMAL NUMBER: 0065
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/17/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4709
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

MISSING TISSUES:
Tibial nerve, Plantar nerve - lost in processing.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0066
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4710
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0067
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/10/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4711
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0068
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4712
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0069
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4713
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0070
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4714
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0071
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4715
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0072
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4716
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0073
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4717
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0074
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4718
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0075
DOSAGE: 500 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4719
GROUP NUMBER: 3
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0076
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/8/78
DEATH: Found dead
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4720
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0077
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4721
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0078
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4722
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0080
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4723
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:

Comment - Appears to be slight muscle wasting of left leg. Representative sections of muscle saved in 10% neutral buffered formalin. No other gross abnormalities noted.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0081
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/22/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4724
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0082
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4725
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - trace nerve fiber degeneration.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0083
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4728
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0084
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4729
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Sciatic nerve - trace nerve fiber degeneration.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0086
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/22/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4730
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0087
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4731
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0089
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4732
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0090
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4733
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0091
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4734
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0092
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4735
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - one lost in processing; the other
no lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0093
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/27/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4736
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0095
DOSAGE: 1000 mg/kg
DATE OF DEATH: 3/19/78
DEATH: Moribund sacrifice
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4737
GROUP NUMBER: 4
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Tibial nerve, Plantar nerve - one lost in processing; the other no
no lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 2J566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0097
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4738
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0100
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4739
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0101
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4740
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0103
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4741
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0105
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4742
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0106
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4743
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0107
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4744
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:

Comment - Nerve at about midway.

MICROSCOPIC FINDINGS:

Tibial nerve, Plantar nerve - one tissue inadequate; the other no lesion recognized.

ANIMAL NUMBER: 0109
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4745
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:

No lesion recognized.

MICROSCOPIC FINDINGS:

No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20566

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0110
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4746
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
Sciatic nerve - slight nerve fiber degeneration.
Tibial nerve, Plantar nerve - minimal nerve fiber degeneration.

ANIMAL NUMBER: 0111
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4747
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

LITTON BIONETICS, INC.
LBI PROJECT NO. 20506

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 0113
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4748
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

ANIMAL NUMBER: 0115
DOSAGE: 1500 mg/kg
DATE OF DEATH: 3/3/78
DEATH: Terminal kill
METHOD OF KILL: Cervical dislocation

PM NUMBER: 78/4749
GROUP NUMBER: 5
SPECIES: Chicken
SEX: Female

GROSS FINDINGS:
No lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

PART I - SECTION G
THREE-MONTH SUBCHRONIC TOXICITY IN DOGS

DIMP

LBI PROJECT NO. 10734-08

SUMMARY

Beagle dogs, four per sex per group, were given diisopropylmethylphosphate (DIMP) in the diet for 90 days. Dietary concentrations were 150, 1500 and 3000 ppm, and a control group was maintained in parallel. Initially and at 4, 8 and 13 weeks hemograms and clinical chemistry values were obtained on all dogs.

The dogs were examined daily as to general condition, and weekly body weights and food consumption data were obtained. An ophthalmologic examination was conducted initially and at 13 weeks. At termination each dog was grossly necropsied and approximately 27 tissues were preserved. Eight organs were weighed. Tissues from the control and high-level groups were examined histologically. The dogs continued in good general health throughout the study. No clear or meaningful changes were seen in the data collected that could be ascribed to the ingestion of DIMP by these dogs, and it is concluded that this compound produced no toxic effects at a dietary concentration of 3000 ppm or below, over the 90-day period of study.

1. OBJECTIVE

The objective of this study was to evaluate and characterize the toxicity of the test material by feeding it in the diet to dogs for 90 days.

2. MATERIAL

Refer to Part I - Section A.

3. EXPERIMENTAL DESIGN

Purebred beagle dogs (five to six months old) were received from Hazleton Research Animals, Inc., Cumberland, Virginia, and acclimated to laboratory conditions for at least four weeks. The dogs had been immunized for hepatitis and rabies by the supplier and were further immunized against distemper and leptospirosis in this laboratory. The dogs were individually housed in stainless steel cages in temperature-controlled quarters with automated artificial lighting providing a 12-hr cycle of illumination. Water was provided ad libitum.

3. EXPERIMENTAL DESIGN (Continued)

The dogs were assigned LBJ dog numbers and placed in the following treatment groups:

<u>Group Number</u>	<u>Animal Number</u>		<u>Diet Concentrations (ppm)</u>
	<u>Males</u>	<u>Females</u>	
1	370-373	374-377	0 (Control)
2	378-381	382-385	150
3	386-389	390-393	1500
4	394-397	398-401	3000

These dietary concentrations were selected by a representative of the sponsor. The dogs were identified by ear tattoo and cage cards.

DIMP was incorporated in the diet of the dogs (Purina Lab Canine Diet) at the stated concentrations using PEG 400 as a vehicle, and mixed with the feed in a twin-shell blender for 20-30 min. The proportion of PEG 400 to feed was 150 ml to 20 kg for all diet levels, including that of the control. Compound feeding began September 2, 1977.

The dogs were examined daily as to general condition, behavior, fecal consistency and signs of toxicity. Body weights were obtained initially and weekly during the experiment. Food consumption was estimated twice per week and recorded on a weekly basis. In addition, fecal examinations for parasites were made on all dogs, and after finding Giardia canis and Isospora canis in some animals, all were treated with sulfamethazine and quinacrine hydrochloride in sequence over a 12-day period.

Clinical pathology determinations were obtained initially and at 4, 8 and 13 weeks. These included:

<u>Hematology</u>	<u>Blood Chemistry</u>	
erythrocyte count	glucose	uric acid
leukocyte count	blood urea nitrogen	bilirubin
differential leukocyte count	serum glutamic-pyruvic transaminase	cholesterol
hemoglobin	serum glutamic-oxaloacetic transaminase	lactic dehydrogenase
packed cell volume	serum alkaline phosphatase	acetyl cholinesterase** (RBC and plasma)
clotting time	total protein	CPK*
	albumin/globulin ratio*	total iron*
	creatinine*	triglycerides*
	sodium*	carbon dioxide*
	chloride*	phosphorus*
	calcium	albumin*
		potassium*

*Initially only except as noted

**Only on dogs receiving DIMP except as noted

Qualitative urinalyses were performed at Weeks 8 and 13.

3. EXPERIMENTAL DESIGN (Continued)

Detailed physical examinations were performed initially and ophthalmologic examinations were performed initially and at 13 weeks, using a solution of 1.0% Mydriacyl to dilate the pupils. (Note: Initial examinations were all made during the acclimation period.)

At 13 weeks the dogs were killed with an intravenous dose of a barbiturate and exsanguinated.

A gross necropsy was performed and the following organs from all dogs were removed and weighed:

liver
brain
thyroid
kidneys
adrenal glands
testes
ovaries
heart
spleen

The weighed organs as well as those listed below were fixed in 10% buffered formalin:

spinal cord	pituitary
lungs	bone marrow (femur)
pancreas	rib junction
stomach	lymph node (mesenteric)
small intestines	mammary tissue
colon	skin
urinary bladder	peripheral nerve (sciatic)
prostate	muscle
eyes (with optic nerve)	uterus
	gallbladder
	any gross lesions

The tissues from dogs of the control and high-level groups were examined histologically while those of the other two groups were stored in case of need.

Thirty days after transmittal of this report, all original data will be transferred to the LBI Archives, 1330 Piccard Drive, Rockville, Maryland.

A draft of this report and underlying data were reviewed by Litton Bionetics Quality Assurance Unit prior to submission.

TABLE I-G-47

LITTON BIOMETICS, INC.
PROJECT NO. 10734-08

TABLE A

FREQUENCY^a OF CLINICAL OBSERVATIONS IN DOGS

WEEK	OBSERVATION	DIET CONCENTRATION - PPM							
		0		150		1500		3000	
		M	F	M	F	M	F	M	F
-1	Soft Feces	2	1		2	2	2	2	1
	Vomiting								1
1	Soft Feces	1			1		1	1	
	Diarrhea	1			1				
2	Soft Feces	3		1	1			3	
	Diarrhea						1		
	Vomiting								1
3	Soft Feces	3	1	2	1	2	2	2	
	Vomiting				1				
	Hair Loss on Foreleg					1			
4	Soft Feces	3	2	1		1	3	2	4
	Vomiting		1						
5	Soft Feces	3	1	2	2	2	1	3	3
	Estrus				1				
	Hair Loss on Foreleg					1			
	Vomiting						1		
6	Soft Feces	4	4	1	3	2	2	3	4
	Watery Feces		1		1	1	2	1	
	Decreased Activity						1		
7	Soft Feces	4	1	3	2	1	2	2	1
	Watery Feces	1	1		1	1			
	Hair Loss on Foreleg					1			
8	Soft Feces	2				1	2	1	2
	Hair Loss on Foreleg					2			1
	Hair Loss on Ear						1		1
	Estrus								1
9	Soft Feces	2	2		2	2	1	1	1
	Watery Feces			1	2	1		1	
	Hair Loss, Sores on Forepaws			1					
	Estrus						1		1
	Hair Loss on Foreleg					2			1
	Hair Loss on Ear						1		
10	Soft Feces	2	1		4	3	2	2	3
	Watery Feces	3					2		
	Hair Loss on Foreleg(s)			1		2			1
	Vomiting						1		
	Hair Loss on Ear						1		
11	Soft Feces	3	1	1	1	2	3	3	3
	Watery Feces	1			1	2		1	
	Hair Loss on Foreleg(s)			1		2			
	Hair Loss on Ear						1		1

^aNumber of dogs showing indicated signs at least once during the week.

TABLE I-G-47 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE A (CONTINUED)

FREQUENCY^a OF CLINICAL OBSERVATIONS IN DOGS

WEEK	OBSERVATION	DIET CONCENTRATION - PPM							
		0		150		1500		3000	
		M	F	M	F	M	F	M	F
12	Soft Feces	3	2	1			1	1	1
	Watery Feces	1	1					1	
	Hair Loss on Foreleg(s)			1		2	1		1
	Hair Loss on Ear						1		
13	Soft Feces	2			1	3	2	2	2
	Watery Feces	1				2	1	1	
	Hair Loss on Foreleg(s)					3			1
	Hair Loss on Ear						1		
	Hair Loss and Sores on Forelegs			1					
	Hair Loss on Abdomen					1			

4. RESULTS (Continued)

No changes in the eyes of any dogs were reported following the ophthalmologic examinations. The only notations given at the terminal examination were for amelanotic chorioid in both eyes of female dog No. 383 (150-ppm group) and in male dog No. 388 (1500-ppm group), both notations accompanied by the comment, "normal variation". Male dog No. 386 (1500-ppm group) was thought to have larger-than-normal optic discs at the terminal examination.

Hematologic data are presented in Appendix Table 4 and group means for the hematocrits, hemoglobin concentrations, erythrocyte counts and leukocyte counts are given in Text Tables B and C for males and females, respectively. No dose-related changes were seen in these measurements throughout the 13 weeks of study.

Clinical chemistry data are given in Appendix Table 5, with summaries for those tests which were followed throughout the study also being given in Text Tables B and C. (Some tests not specified in the protocol were done initially as part of routine screening procedures and are shown in Table 5. Since these tests were not repeated, their means are not summarized in Text Tables B and C.)

No dose- or time-related changes were apparent with respect to glucose, blood urea nitrogen, SGOT, SGPT, alkaline phosphatase, lactic dehydrogenase, calcium, phosphorus, total protein, albumin, bilirubin, uric acid or cholesterol. Values for some of these tests varied markedly, but statistical evaluations failed to show significance in any consistent or meaningful way.

Clotting time means (Appendix Table 6, Text Tables B and C) likewise showed no pattern indicative of compound effect. Only one of these means was statistically significantly different from control mean, that of the 1500-ppm males at 4 weeks.

Plasma and RBC cholinesterase data are given in Appendix Table 5, with summaries of group means in Text Tables B and C. As preface to discussion of these data, it may be noted that a misinterpretation of the wording of the experimental protocol led to the omission of cholinesterase assays for the control dogs at Weeks 4 and 8, and while the enzyme was determined in all eight controls initially, technical difficulties with the RBC samples at Week 0 make all values for RBC invalid for that time interval. (Plasma values were not affected.) Moreover, at Week 13 cholinesterase for only two control dogs was assayed. Thus, attempts to perceive a pattern in RBC and plasma cholinesterases are somewhat complicated.

TABLE I-G-48

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-08

TABLE B

MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES IN MALE DOGS

	DIET CONCENTRATIONS (PPM)	INTERVAL (WEEKS)			
		0	4	8	13
PCV (%)	0	48	48	48	49
	150	44	50	48	47
	1500	42*	48	48	47
	3000	44	46	48	49
HGB (g %)	0	15.7	16.9	16.9	16.6
	150	14.8	17.2	16.9	15.8
	1500	14.2	17.1	17.1	15.6
	3000	15.0	15.9	17.6	16.4
RBC/ MM ³ x 10 ⁶	0	7.54	6.91	8.46	7.21
	150	6.57	7.00	8.07	7.11
	1500	6.49	6.95	7.88	7.23
	3000	6.63	6.82	7.71	7.60
WBC/ MM ³ x 10 ³	0	7.7	9.1	12.3	11.0
	150	7.9	10.3	14.5	10.6
	1500	6.5	10.9	13.5	10.7
	3000	7.6	9.9	11.2	9.4
CLOTTING TIME (SEC)	0	353	405	488	458
	150	375	555	488	570
	1500	465	675*	533	518
	3000	435	578	488	480
GLUCOSE (MG/DL)	0	99	97	88	89
	150	120	109	93	99
	1500	94	109	93	104
	3000	111	106	101	105
BUN (MG/DL)	0	12	15	18	14
	150	10	14	15	14
	1500	14	14	14	14
	3000	10	12	13	13
SGOT (MIU/ML)	0	40	37	35	45
	150	40	41	46	52
	1500	36	37	45	50
	3000	31	32	40	49

*p<0.05 as compared to controls: Dunnett's t-test.

TABLE I-G-48 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE B (CONTINUED)

MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES IN MALE DOGS

	DIET CONCENTRATIONS (PPM)	INTERVAL (WEEKS)			
		0	4	8	13
SGPT (MIU/ML)	0	32	41	38	44
	150	37	36	35	44
	1500	29	35	36	36
	3000	32	42	42	44
ALK. PHOS. (MIU/ML)	0	152	127	124	92
	150	112	87	77	53
	1500	113	84	74	51
	3000	106	72	71	48
LDH (MIU/ML)	0	225	87	183	231
	150	238	98	287	518
	1500	213	83	269	499
	3000	158	53	201	332
CALCIUM (MG/DL)	0	11.9	11.3	10.8	11.2
	150	11.6	11.4	10.8	10.9
	1500	11.8	11.4	10.6	11.2
	3000	11.4	11.3	10.5	11.0
PHOSPHORUS (MG/DL)	0	6.8	4.9	5.4	5.1
	150	6.0	4.6	4.8*	4.5
	1500	6.3	4.5	4.7*	4.6
	3000	6.4	4.4	4.7*	4.6
TOTAL PROTEIN (G/DL)	0	6.3	6.3	6.9	6.6
	150	5.8	5.9	6.6	6.3
	1500	6.0	6.1	6.6	6.6
	3000	5.8	6.0	6.5	6.4
ALBUMIN (G/DL)	0	3.5	3.5	3.2	3.3
	150	3.2	3.5	3.1	3.3
	1500	2.9	3.7	3.1	3.4
	3000	2.9	3.5	3.1	3.3
BILIRUBIN (MG/DL)	0	0.2	0.1	0.4	0.1
	150	0.3	0.1	0.4	0.1
	1500	0.3	0.1	0.1	0.4
	3000	0.3	0.1	0.1	0.2

* $p < 0.05$ as compared to controls: Dunnett's t-test.

TABLE I-G-48 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-08

TABLE B (CONTINUED)

MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES IN MALE DOGS

	DIET CONCENTRATIONS (PPM)	INTERVAL (WEEKS)			
		0	4	8	13
URIC ACID (MG/DL)	0	0.4	0.4	0.4	0.6
	150	0.3	0.8	0.6	1.1
	1500	0.2	0.3	0.5	0.9
	3000	0.3	0.3	0.5	0.6
CHOLESTEROL (MG/DL)	0	182	164	176	156
	150	163	141	152	145
	1500	167	167	166	170
	3000	169	153	152	142
PLASMA CHOLIN - ESTERASE (MU/ML)	0	2359	--	--	2704 ^a
	150	2062	1862	2176	1888
	1500	1775	1571	1692	1687
	3000	2087	1750	1798	1839
RBC CHOLIN - ESTERASE (MU/ML)	0	685	---	--	2688 ^a
	150	798	2667	2688	2302
	1500	815	4804	3181	1982
	3000	523	4889	2709	3392

^aMean of 2 dogs.

TABLE I-G-49

TABLE C

MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES IN FEMALE DOGS

	DIET CONCENTRATION (PPM)	INTERVAL (WEEKS)			
		0	4	8	13
PCV (%)	0	45	49	49	50
	150	46	48	48	46
	1500	48	50	50	49
	3000	45	49	49	48
HGB (G %)	0	15.2	17.2	17.8	16.9
	150	15.1	16.9	17.0	15.5
	1500	16.4	17.3	17.7	16.6
	3000	15.1	17.3	17.7	16.5
RBC/ $\text{MM}^3 \times 10^6$	0	6.84	7.04	8.14	7.38
	150	6.58	6.84	7.87	7.02
	1500	7.09	6.90	8.05	7.43
	3000	6.84	7.07	8.10	7.32
WBC/ $\text{MM}^3 \times 10^3$	0	5.7	9.8	11.7	9.8
	150	6.4	9.8	12.4	10.2
	1500	6.7	9.5	10.8	11.3
	3000	7.6	7.7	14.1	9.8
CLOTTING TIME (SEC)	0	338	465	413	458
	150	443	555	495	638
	1500	443	668	570	540
	3000	465	600	480	600
GLUCOSE (MG/DL)	0	108	109	92	88
	150	109	105	91	101
	1500	113	118	103	103
	3000	122	112	102	104
BUN (MG/DL)	0	11	13	15	13
	150	11	14	15	14
	1500	12	15	16	15
	3000	13	14	15	15
SGOT (MIU/ML)	0	36	37	39	42
	150	32	37	34	54
	1500	30	39	42	51
	3000	33	30	44	46

TABLE I-G-49 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE C (CONTINUED)

MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES IN FEMALE DOGS

	DIET CONCENTRATION (PPM)	INTERVAL (WEEKS)			
		0	4	8	13
SGPT (MIU/ML)	0	27	31	34	35
	150	32	38	41	38
	1500	33	37	40	41
	3000	37	38	46	43
ALK. PHOS. (MIU/ML)	0	110	95	96	63
	150	107	92	96	69
	1500	99	81	74	60
	3000	103	73	76	50
LDH (MIU/ML)	0	269	83	175	232
	150	160	105	175	620
	1500	156	54	234	422
	3000	182	46	256	362
CALCIUM (MG/DL)	0	11.9	11.4	11.0	11.1
	150	11.8	11.5	10.8	11.1
	1500	11.8	11.7	11.1	11.4
	3000	11.6	11.3	10.6	10.9
PHOSPHORUS (MG/DL)	0	6.2	4.3	4.6	4.4
	150	6.5	4.5	4.8	4.5
	1500	6.1	4.7	4.5	4.5
	3000	6.2	4.5	4.5	4.6
TOTAL PROTEIN (G/DL)	0	6.1	6.2	6.7	6.4
	150	5.8	6.1	6.4	6.3
	1500	6.0	6.0	6.8	6.5
	3000	5.8	5.7	6.6	6.4
ALBUMIN (G/DL)	0	3.4	3.7	3.3	3.4
	150	3.1	3.7	3.2	3.4
	1500	3.1	3.7	3.3	3.1
	3000	2.9	3.5	3.2	3.3
BILIRUBIN (MG/DL)	0	0.4	0.1	0.4	0.1
	150	0.3	0.1	0.4	0.1
	1500	0.2	0.1	0.1	0.3
	3000	0.2	0.1	0.1	0.2

TABLE I-G-49 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-08

TABLE C (CONTINUED)

MEAN HEMATOLOGICAL AND CLINICAL CHEMISTRY VALUES IN FEMALE DOGS

	DIET CONCENTRATION (PPM)	INTERVAL (WEEKS)			
		0	4	8	13
URIC ACID (MG/DL)	0	0.3	0.5	1.2	0.5
	150	0.2	0.5	0.5	0.8
	1500	0.3	0.7	0.4	0.6
	3000	0.2	0.3	0.4	0.5
CHOLESTEROL (MG/DL)	0	188	165	175	158
	150	177	180	191	215
	1500	176	162	173	186
	3000	174	151	166	173
PLASMA CHOLIN - ESTERASE (MU/ML)	0	2324	--	--	1779 ^a
	150	1820	1739	1890	1919
	1500	2296	2003	2257	2284
	3000	2016	1603	1846	1779
RBC CHOLIN - ESTERASE (MU/ML)	0	1075	--	--	2431 ^a
	150	865	3772	2919	2302
	1500	1018	4576	2478	2045
	3000	735	4016	2281	2045

^aMean of 2 dogs.

4. RESULTS (Continued)

For plasma, if one considers each group mean at Week 0 for the DIMP-dosed dogs as the index of enzyme activity (100%), there seemed to be a tendency toward slight inhibition in the 3000-ppm group (16-20% at Week 4, but only 8-14% at Week 13), while dogs of the other two groups showed at most only 13% inhibition at 4 weeks. Or, to take the 13-week values alone (with the two controls of each sex that were assayed as 100%), the males were inhibited to about the same extent (30-38%) in all three groups, while the females were not inhibited at all. For the RSC enzyme the situation is complicated by the invalid Week 0 values, but if the Week 4 mean of each group is used as the index, there appears to be as much as 45% inhibition for both sexes in the high-dose dogs. If the 13-week means are considered alone, with only two control dogs per sex as 100% activity, there was no clear dose-related effect.

All in all, it is believed that no valid effect on cholinesterase in these dogs was induced by DIMP ingestion.

Appendix Table 7 shows results of qualitative urinalyses. These data are unexceptional and indicate no compound-induced effects.

Absolute organ weights and relative organ weights (organ-to-body weight percentages) are given in Appendix Tables 8 and 9, respectively. Group means of organ weights and relative organ weights are collected in Text Table D. None of the mean values of the 3000-ppm group were statistically significantly different from control mean values. Only the mean relative ovary weight in the group that received 1500-ppm of DIMP in the diet was statistically significantly different from the control mean ($p < 0.05$). The mean absolute ovary weight in this group was not statistically different from control value, however. Since the mean ovary weights, absolute and relative, of the other dose groups were not statistically significantly different from control figures, the difference noted for the 1500-ppm group is not believed to be meaningful.

The report of the histopathologist is appended. Most of the changes seen were such as are commonly encountered in dogs and the only one thought to be possibly associated with compound administration was the occurrence of cystic crypts of Lieberkühn in the small intestines of male dogs No. 395 and No. 397 (both in the 3000-ppm group). Such crypts are thought to be indicative of intestinal hypermotility, and review of daily observations shows these two dogs did have soft or watery feces one or more times per week during much of the study. However, control male No. 370 also had cystic crypts of Lieberkühn and soft feces during the study, and various other dogs had soft feces but no histological intestinal changes. The cystic crypt observations may therefore be regarded as suggestive at most.

LITTON BIONETICS, INC.
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TABLE I-G-50

TABLE D

MEAN ORGAN WEIGHTS

MEAN ABSOLUTE ORGAN WEIGHTS (G)

<u>DOSE</u> <u>(PPM)</u>	<u>BODY</u> <u>WEIGHT</u> <u>(KG)</u>	<u>BRAIN</u>	<u>HEART</u>	<u>LIVER</u>	<u>SPLEEN</u>	<u>KIDNEYS</u>	<u>THYROIDS</u>	<u>ADRENALS</u>	<u>TESTES/</u> <u>OVARIES</u>
<u>MALES</u>									
0	11.5	75.45	90.44	296.2	61.89	57.49	0.88	0.93	22.09
150	12.4	78.87	88.10	314.6	63.54	59.18	0.95	0.96	23.78
1500	11.9	80.40	86.79	314.6	63.49	60.05	1.00	1.05	22.59
3000	10.6	79.65	85.77	296.4	56.38	62.04	0.86	0.97	18.40
<u>FEMALES</u>									
0	10.5	73.32	81.58	262.4	58.06	50.14	0.84	0.97	0.73
150	10.1	75.19	74.88	283.8	50.06	50.39	0.89	1.03	1.04
1500	10.5	70.43	78.85	283.2	55.76	46.13	0.77	1.10	1.95
3000	10.1	73.58	74.69	276.0	69.12	45.29	0.83	0.88	1.46

TABLE I-G-50 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE D (CONTINUED)

MEAN ORGAN WEIGHTS

MEAN RELATIVE ORGAN WEIGHTS (G OR MG/100 G OF BODY WEIGHT)

<u>DOSE</u> <u>(PPM)</u>	<u>BRAIN</u>	<u>HEART</u>	<u>LIVER</u>	<u>SPLEEN</u>	<u>KIDNEYS</u>	<u>THYROIDS</u>	<u>ADRENALS</u>	<u>TESTES/</u> <u>OVARIES</u>
<u>MALES</u>								
0	0.657	0.783	2.575	0.549	0.492	7.77	8.18	0.192
150	0.643	0.713	2.560	0.511	0.480	7.71	7.80	0.193
1500	0.679	0.731	2.661	0.535	0.509	8.48	8.83	0.191
3000	0.733	0.785	2.721	0.517	0.570	8.06	8.79	0.168
<u>FEMALES</u>								
0	0.713	0.786	2.514	0.549	0.485	8.08	9.36	7.12
150	0.752	0.743	2.829	0.496	0.505	8.75	10.24	9.95
1500	0.674	0.751	2.689	0.527	0.439	7.27	10.38	18.27*
3000	0.744	0.746	2.776	0.692	0.449	8.34	8.85	14.58

*p<0.05 compared to controls: Student's t-test.

5. CONCLUSION

Based on the results presented it is concluded that no dose- or time-related toxic effects were produced in dogs given DIMP in the diet at concentrations of 150, 1500 and 3000 ppm daily for 90 days.

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TABLE I-G-51

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE 1

BODY WEIGHT (KG)

GROUP 1 - 0 PPM

ANIMAL
NO.

WEEK

	1	2	3	4	5	6	7	8	9	10	11	12	13
MALES													
370	8.6	8.6	8.8	9.4	9.2	9.4	9.4	9.6	9.8	10.8	9.8	9.8	10.2
371	9.8	9.8	10.0	10.0	10.2	10.2	10.6	11.2	10.8	11.6	10.8	10.9	11.5
372	10.6	10.6	11.2	10.6	10.8	10.6	11.2	12.0	11.8	12.0	11.6	11.6	11.0
373	11.8	12.0	11.2	12.4	12.2	12.4	12.2	12.8	12.8	13.2	12.8	12.8	13.4
MEAN	10.2	10.1	10.3	10.6	10.6	10.7	10.9	11.4	11.3	11.9	11.3	11.3	11.5
SD	1.35	1.12	1.15	1.30	1.25	1.27	1.17	1.37	1.29	1.00	1.27	1.26	1.36
SE	0.67	0.56	0.57	0.65	0.63	0.63	0.59	0.68	0.65	0.50	0.63	0.63	0.68
FEMALES													
374	7.2	7.3	7.4	7.8	7.6	7.6	7.8	8.2	8.0	8.8	8.2	8.2	8.2
375	8.6	8.6	8.8	8.8	8.8	9.0	9.2	9.2	9.2	10.0	9.8	9.8	9.3
376	10.2	10.2	9.8	9.8	10.2	10.4	10.4	11.0	10.6	11.2	11.2	11.4	10.8
377	10.0	10.2	10.4	10.6	10.8	11.0	-	11.2	11.2	11.8	11.8	12.1	12.0
MEAN	9.0	9.1	9.1	9.3	9.4	9.5	9.1	9.9	9.8	10.5	10.3	10.4	10.1
SD	1.40	1.40	1.31	1.22	1.44	1.52	1.30	1.45	1.44	1.33	1.60	1.74	1.67
SE	0.70	0.70	0.66	0.61	0.72	0.75	0.75	0.72	0.72	0.67	0.80	0.87	0.83

* Fasted 1 night for bloods.

TABLE I-G-51 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE 1 (CONTINUED)

BODY WEIGHT (KG)

GROUP 2 - 150 PPM

ANIMAL NO.	WEEK												
	1	2	3	4	5	6	7	8 ^a	9	10	11	12	13 ^a
MALES													
378	10.6	10.6	10.4	10.4	10.6	10.6	10.6	11.0	10.8	11.8	11.6	11.4	11.5
379	10.6	10.8	10.2	10.8	11.0	11.0	11.2	12.2	11.4	11.8	11.4	11.6	11.9
380	11.2	11.3	11.4	11.4	11.8	11.8	12.4	12.6	12.8	13.8	13.0	13.1	13.2
381	11.0	11.0	11.0	11.4	11.2	11.4	11.2	11.8	12.4	13.2	12.2	12.2	12.8
MEAN	11.1	11.1	11.0	11.0	11.2	11.2	11.4	11.9	11.9	12.7	12.1	12.1	12.4
SD	0.57	0.54	0.77	0.49	0.50	0.52	0.75	0.68	0.91	1.01	0.72	0.76	0.79
SE	0.29	0.27	0.39	0.24	0.25	0.26	0.38	0.34	0.46	0.51	0.36	0.38	0.39
FEMALES													
382	8.2	8.0	8.4	8.6	8.8	8.6	8.8	8.2	8.8	10.2	9.2	9.5	9.0
383	8.2	8.1	8.2	8.2	8.4	8.4	8.6	8.8	9.2	9.4	8.6	8.6	8.8
384	9.8	9.8	9.8	10.6	10.2	10.6	11.4	11.2	11.2	12.0	11.4	11.4	11.1
385	10.6	10.8	10.8	11.0	11.2	11.4	11.8	12.2	11.8	12.2	11.2	11.2	11.5
MEAN	9.2	9.2	9.3	9.6	9.7	9.8	10.2	10.1	10.3	11.0	10.1	10.2	10.1
SD	1.20	1.36	1.23	1.40	1.29	1.48	1.68	1.91	1.47	1.37	1.41	1.35	1.40
SE	0.60	0.68	0.61	0.70	0.64	0.74	0.84	0.95	0.74	0.68	0.70	0.68	0.70

^a Fasted 1 night for bloods.

TABLE I-G-51 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE 1 (CONTINUED)

BODY WEIGHT (KG)

GROUP 3 - 1500 PPM

ANIMAL NO.	WEEK												
	1	2	3	4	5	6	7	8 ^a	9	10	11	12	13 ^a
MALES													
386	9.6	10.6	10.2	8.4	10.4	10.6	11.6	10.2	10.8	11.8	11.2	11.3	11.0
387	10.4	10.3	10.4	10.6	10.6	11.2	11.4	11.2	11.6	11.8	11.6	11.7	11.6
388	11.2	11.4	11.4	11.8	11.6	11.8	11.8	12.4	12.2	12.8	12.8	13.0	12.5
389	11.6	11.8	11.6	11.8	12.2	12.2	11.8	12.2	11.6	12.8	12.2	12.1	12.3
MEAN	10.7	11.0	10.9	10.7	11.2	11.5	11.7	11.5	11.6	12.3	12.0	12.0	11.9
SD	0.89	0.67	0.70	1.60	0.85	0.70	0.19	1.01	0.57	0.58	0.70	0.73	0.69
SE	0.44	0.33	0.35	0.80	0.42	0.35	0.10	0.51	0.29	0.29	0.35	0.36	0.34
FEMALES													
390	8.4	8.2	8.6	8.8	9.0	9.2	9.2	9.6	9.8	10.2	10.2	10.2	10.2
391	8.4	8.6	8.8	9.0	9.4	9.2	9.0	9.8	9.4	10.0	10.0	10.1	9.6
392	8.6	8.8	8.6	9.0	9.2	9.6	9.8	10.2	10.2	10.8	10.2	10.4	10.2
393	10.2	10.3	10.8	10.8	10.8	10.8	11.4	11.4	11.4	12.2	11.6	11.7	12.1
MEAN	8.9	9.0	9.2	9.4	9.6	9.7	9.9	10.3	10.2	10.8	10.5	10.6	10.5
SD	0.87	0.92	1.07	0.94	0.82	0.76	1.09	0.81	0.86	0.99	0.74	0.74	1.09
SE	0.44	0.46	0.54	0.47	0.41	0.38	0.54	0.40	0.43	0.50	0.37	0.37	0.54

^aFasted 1 night for bloods.

TABLE I-G-51 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE 1 (CONTINUED)

BODY WEIGHT (KG)

GROUP 4 - 3000 PPM

ANIMAL NO.	WEEK												
	1	2	3	4	5	6	7	8	9	10	11	12	13 ^a
MALES													
394	9.8	10.1	9.6	10.2	9.8	10.2	10.2	10.4	10.6	11.2	10.6	10.9	10.9
395	9.2	9.3	9.4	9.6	9.8	9.6	9.8	9.8	10.2	10.4	9.8	10.0	9.4
396	11.0	11.1	11.2	11.0	11.2	11.2	11.2	11.4	11.6	12.4	11.6	11.6	11.2
397	11.2	11.2	11.8	12.0	11.8	11.8	12.2	12.2	12.2	13.2	12.8	12.8	12.3
MEAN	10.3	10.4	10.5	10.7	10.7	10.7	10.9	11.0	11.2	11.8	11.2	11.3	11.0
SD	0.96	0.90	1.18	1.04	1.01	0.99	1.10	1.06	0.91	1.24	1.30	1.18	1.20
SE	0.48	0.45	0.59	0.52	0.51	0.49	0.55	0.53	0.46	0.62	0.65	0.59	0.60
FEMALES													
398	7.0	7.2	7.4	7.6	7.8	7.8	8.2	8.2	8.2	9.2	8.2	8.3	8.6
399	8.8	8.8	8.8	8.8	8.6	8.8	9.2	9.4	9.2	9.4	9.8	9.8	9.2
400	9.8	9.7	9.8	10.2	10.2	10.2	10.4	8.8	10.4	11.6	11.2	11.2	11.1
401	10.6	10.8	10.8	11.0	10.8	11.0	11.2	11.4	11.0	11.8	11.6	11.6	11.3
MEAN	9.1	9.1	9.2	9.4	9.4	9.5	9.8	9.5	9.7	10.5	10.2	10.2	10.1
SD	1.55	1.52	1.45	1.51	1.39	1.43	1.32	1.39	1.25	1.39	1.54	1.50	1.35
SE	0.78	0.76	0.73	0.75	0.69	0.71	0.66	0.69	0.62	0.69	0.77	0.75	0.68

^a Fasted 1 night for bloods.

TABLE I-G-52

LITTON BIONUTRICS, INC.
PROJECT NO. 10734-08

TABLE 2

FOOD CONSUMPTION (KG)

GROUP 1 - 0 PPM

ANIMAL NO.	WEEK												
	1	2	3 ^a	4	5	6	7	8	9	10	11	12	13
MALES													
370	3.4	2.4	-	2.1	2.7	3.0	3.3	3.0	3.8	2.8	2.5	2.5	2.9
371	2.5	2.3	-	1.9	2.4	2.7	3.2	2.0	2.6	2.9	2.5	2.7	3.1
372	3.2	S.F.	-	2.8	S.F.	2.2	2.8	2.4	3.0	2.5	1.8	2.2	2.0
373	2.3	2.3	-	2.0	2.3	2.9	2.2	2.4	2.4	2.6	2.9	2.7	2.4
MEAN	2.9	2.3		2.2	2.5	2.7	2.9	2.7	3.0	2.7	2.4	2.5	2.6
SD	0.53	0.06		0.41	0.21	0.36	0.50	0.30	0.62	0.18	0.46	0.24	0.50
SE	0.27	0.03		0.20	0.12	0.18	0.25	0.15	0.31	0.09	0.23	0.12	0.25
FEMALES													
374	1.8	2.0	-	1.6	1.9	2.1	2.0	2.7	2.0	2.1	S.F.	1.9	2.1
375	1.6	1.7	-	1.5	S.F.	2.3	2.1	2.1	2.4	2.4	1.8	2.4	1.9
376	2.2	2.1	-	1.6	1.6	2.6	2.4	2.5	2.7	2.5	2.5	2.3	2.4
377	1.8	1.2	-	2.0	2.3	3.0	2.4	2.4	2.3	2.2	2.4	2.4	2.3
MEAN	1.9	1.8		1.7	1.9	2.5	2.2	2.4	2.4	2.3	2.2	2.3	2.2
SD	0.25	0.40		0.22	0.35	0.39	0.21	0.25	0.29	0.18	0.38	0.24	0.22
SE	0.13	0.20		0.11	0.20	0.20	0.10	0.13	0.14	0.09	0.22	0.12	0.11

^aNot measured Week 3: sm text.

TABLE I-G-52 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-08

TABLE 2 (CONTINUED)

FOOD CONSUMPTION (KG)

GROUP 2 - 150 PPM

ANIMAL NO.	1	2	3	4	5	6	7	8	9	10	11	12	13
MALES													
378	2.4	S.F.	-	S.F.	S.F.	2.4	2.3	1.9	2.4	2.7	2.4	2.0	2.2
379	S.F.	S.F.	-	S.F.	S.F.	3.3	3.6	2.6	3.0	2.4	2.6	3.1	S.F.
380	S.F.	S.F.	-	S.F.	S.F.	3.4	4.1	3.9	S.F.	3.5	3.4	S.F.	3.9
381	S.F.	S.F.	-	1.7	2.0	2.3	2.5	2.5	2.9	2.5	2.2	2.6	2.7
MEAN						2.9	3.1	2.7	2.8	2.8	2.7	2.5	2.9
SD						0.58	0.87	0.84	0.32	0.50	0.53	0.57	0.87
SE						0.29	0.43	0.42	0.19	0.25	0.26	0.33	0.50
FEMALES													
382	2.0	2.1	-	1.6	2.1	2	2.3	1.9	2.5	2.4	2.1	2.2	2.7
383	1.5	1.4	-	1.1	1.1	2	1.4	1.5	2.0	1.8	1.6	1.7	2.2
384	S.F.	2.2	-	1.9	S	2	S.F.	2.3	2.7	2.0	2.4	2.6	2.4
385	2.2	2.4	-	2.0	2	2	2.5	2.6	2.6	1.9	2.1	2.3	1.9
MEAN	1.9	2.0		1.7	2.1	2.1	2.1	2.1	2.5	2.2	2.1	2.2	2.3
SD	0.51	0.75		0.70	0.85	0.85	0.59	0.48	0.31	0.46	0.33	0.37	0.34
SE	0.29	0.38		0.35	0.49	0.49	0.34	0.24	0.16	0.23	0.17	0.19	0.17

*Not measured Week 3: see text.

TABLE I-G-52 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-00

TABLE 2 (CONTINUED)

FOOD CONSUMPTION (KG)

GROUP 3 - 1500 PPM

ANIMAL NO.	WEEK	1	2	3 ^a	4	5	6	7	8	9	10	11	12	13
MALES														
386	2.7	2.7	-	-	2.6	S.F.	2.2	2.2	1.6	2.1	2.5	2.1	2.1	2.2
387	2.4	2.3	-	-	1.9	S.F.	1.9	2.1	2.3	2.6	2.3	2.5	2.0	2.1
388	S.F.	1.9	-	-	1.8	S.F.	2.5	1.8	2.3	2.4	2.1	2.2	2.4	2.1
389	2.5	2.4	-	-	1.7	2.2	2.2	1.7	1.4	2.0	2.1	2.2	2.3	2.5
MEAN	2.5	2.3	-	-	2.0	-	2.2	2.0	1.9	2.3	2.3	2.3	2.2	2.1
SD	0.15	0.33	-	-	0.41	-	0.24	0.24	0.47	0.28	0.19	0.17	0.18	0.19
SE	0.09	0.17	-	-	0.20	-	0.12	0.12	0.23	0.14	0.10	0.09	0.09	0.09
FEMALES														
390	1.9	1.5	-	-	2.0	2.2	2.0	2.0	1.9	2.7	2.4	2.5	2.5	2.5
391	S.F.	1.8	-	-	1.7	2.0	1.9	1.9	1.8	2.2	2.1	1.9	2.1	1.9
392	3.1	S.F.	-	-	S.F.	S.F.	2.3	2.1	2.1	2.4	2.3	3.2	2.4	2.2
393	S.F.	2.0	-	-	1.7	2.1	2.4	2.4	2.0	2.2	2.0	2.1	2.6	1.9
MEAN	2.5	1.8	-	-	1.8	2.1	2.2	2.2	2.0	2.4	2.2	2.4	2.4	2.1
SD	0.85	0.25	-	-	0.17	0.10	0.26	0.24	0.13	0.24	0.18	0.57	0.22	0.29
SE	0.60	0.15	-	-	0.10	0.06	0.13	0.12	0.06	0.12	0.09	0.29	0.11	0.14

^aNot measured Week 3: see text.

TABLE I-G-52 (Continued)

LITTON DIONETICS, INC.
PROJECT NO. 10734-08

TABLE 2 (CONTINUED)

FOOD CONSUMPTION (KG)

GROUP 4 - 3000 PPM

ANIMAL NO.	WEEK												
	1	2	3 ^a	4	5	6	7	8	9	10	11	12	13
MALES													
394	2.2	-	-	2.0	2.4	2.4	2.4	2.4	2.7	2.2	2.6	S.F.	2.0
395	1.6	1.6	-	1.4	1.8	1.7	1.9	1.6	1.7	1.7	1.5	1.9	2.2
396	2.7	2.2	-	S.F.	S.F.	2.2	2.3	2.2	2.6	2.5	S.F.	2.4	2.2
397	1.8	2.2	-	2.3	2.4	2.4	2.1	2.0	2.6	3.1	2.5	2.8	S.F.
MEAN	2.1	2.0	-	1.9	2.2	2.2	2.2	2.1	2.4	2.4	2.2	2.4	2.1
SD	0.49	0.35	-	0.46	0.35	0.33	0.22	0.34	0.47	0.59	0.61	0.45	0.12
SE	0.24	0.20	-	0.26	0.20	0.17	0.11	0.17	0.23	0.29	0.35	0.26	0.07
FEMALES													
398	1.4	1.9	-	1.6	2.1	2.1	2.1	1.9	1.8	2.2	2.1	2.4	2.0
399	1.5	2.3	-	1.3	1.4	1.9	1.8	1.8	1.6	2.1	1.7	2.1	2.0
400	2.1	1.9	-	1.8	1.9	2.3	1.7	2.2	2.3	2.2	2.7	2.9	2.2
401	3.1	2.4	-	2.2	2.6	2.5	2.7	1.8	2.7	2.2	2.5	2.7	2.1
MEAN	2.0	2.1	-	1.7	2.0	2.2	2.1	1.9	2.1	2.2	2.3	2.5	2.1
SD	0.78	0.26	-	0.38	0.50	0.26	0.45	0.19	0.50	0.05	0.44	0.35	0.10
SE	0.39	0.13	-	0.19	0.25	0.13	0.23	0.09	0.25	0.03	0.22	0.18	0.05

^aNot measured Week 3: see text.

THREE MONTH TOXICITY STUDY IN DOGS

LBI PROJECT NO. 10754-08

HISTOPATHOLOGIC SUMMARY

A summary of the significant histologic observations in this group of eight high-dose and eight control dogs is appended.

The cystic crypts of Lieberkuhn noted in #395 (3000 ppm) and #397 (3000 ppm) may have been related to oral dosing with this compound. Such changes were not noted in the controls. Cystic crypts are considered to be an indication of intestinal hypermotility, which may have been manifested clinically as diarrhea prior to death. Cystic crypts are a result of diarrhea, not the cause of diarrhea. The cysts were filled with cell debris and none had progressed to the "crypt abscess" stage, therefore the lesion was considered to be mild and of short duration.

The observed hypospermatogenesis was more pronounced in #397 (3000 ppm) than in #373 (0 ppm), but the changes were rather mild in both dogs. Lack of spermatogenesis in a few seminiferous tubules is commonly observed in dogs and is not considered to be particularly significant. Examination of a very large number of dogs would be required to determine whether there was a significant increase in the incidence or severity of this change in dosed dogs. Results from the present group of sixteen dogs do not suggest such an effect.

The remaining lesions are relatively common incidental findings or spontaneous diseases of dogs. The most significant of these was the nonsuppurative thyroiditis noted in #400 (3000 ppm). The lesion was very pronounced in one thyroid lobe and milder in the other lobe. There were multiple nodular aggregations of lymphocytes among the moderate diffuse infiltration of lymphocytes and plasma cells. A few thyroid follicles were compromised by the disease process. This spontaneous lesion occurs primarily in beagles and is presently considered to be an immune-mediated disease.

The focal ulcerative dermatitis in #401 (3000 ppm) probably resulted from minor trauma sustained in the kennel or during handling. An etiologic agent was not observed in the H&E stained sections; contamination by multiple bacterial species is suspected.

Cystic Rathke's pouch remnants in the pituitary and cystic ultimobranchial remnants in the parathyroid/thyroid are common incidental findings in dogs. Such small cysts as were observed in these animals are not considered to be of pathologic significance.

Chronic inflammation of the interstitium of the kidney is commonly seen in dogs and often becomes clinically significant in older dogs. The etiology is speculative. The mild changes seen in the kidney of #374 (0 ppm) were considered to be an early manifestation of that disease process.

Cystic glandular hyperplasia of the prostate is nearly ubiquitous in old dogs. The changes noted in the prostate of #396 (3000 ppm) were very mild.

Phagocytosis of erythrocytes by cells of the fixed reticuloendothelial system in the lymph nodes is commonly observed in dogs. The phagocytized erythrocytes in the lymph nodes of #394 (3000 ppm) and #371 (0 ppm) were well preserved and there was no accumulation of hemosiderin. The peracute erythrophagocytosis observed in these two dogs was considered to be an insignificant agonal event.

The genesis of the intra-alveolar hyaline bodies such as were noted in #399 (3000 ppm) is not known. Very large accumulations of such material cause little or no signs of respiratory distress, therefore the minimal accumulation in #399 was considered to be an incidental finding of no pathologic significance.

A number of very minor histologic observations are recorded on the histopathologic records.

In summary, the only histologic change which could reasonably be related to the dosing was the occurrence of cystic crypts in the small intestine. This findings suggests a very mild gastrointestinal disturbance, which may have been clinically manifested as diarrhea. The lesions were very mild and would be expected to resolve within 4-7 days following the termination of the gastrointestinal disturbance.

3 March 1978
Date

George A. Parker, D.V.M.
Veterinary Pathologist

THREE MONTH TOXICITY STUDY IN DOGS

LBI PROJECT NO. 10734-08

SUMMARY OF SIGNIFICANT HISTOLOGIC FINDINGS

- A. Thyroid - nonsuppurative thyroiditis, #400 (3000 ppm).
- B. Small intestine - cystic crypts, #395 (3000 ppm) and #397 (3000 ppm).
- C. Skin - focal ulcerative dermatitis, #401 (3000 ppm).
- D. Parathyroid/thyroid - cystic ultimobranchial remnants, #372 (0 ppm), #395 (3000 ppm) and #397 (3000 ppm).
- E. Kidneys - mild nonsuppurative pyelitis - #374 (0 ppm).
- F. Mesenteric lymph node - erythrophagocytosis, #394 (3000 ppm) and #371 (0 ppm).
- G. Prostate - mild cystic glandular hyperplasia, #396 (3000 ppm).
- H. Pituitary - cystic Rathke's pouch remnant, #394 (3000 ppm) and #371 (0 ppm).
- I. Testis - hypospermatogenesis, #397 (3000 ppm) and #373 (0 ppm).
- J. Lungs - intra-alveolar hyaline bodies - #399 (3000 ppm).

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 370
DOSAGE: 0 ppm
DATE OF DEATH: 12/5/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL: _____

PROJECT NUMBER: 10734
PM NUMBER: 77/11148
GROUP NUMBER: 1
SEX: Male
WEEK OF STUDY: _____

GROSS FINDINGS:
No gross lesion recognized.

MICROSCOPIC FINDINGS:
Mesenteric lymph node - active appearing node.
Small intestine - cystic crypt, duodenum.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 371
DOSAGE: 0 ppm
DATE OF DEATH: 12/6/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethal
MORIBUND KILL: _____

PROJECT NUMBER: 10734-08
PM NUMBER: 77/11156
GROUP NUMBER: 1
SEX: Male
WEEK OF STUDY: _____

GROSS FINDINGS:

No gross lesions recognized.

MICROSCOPIC FINDINGS:

Thyroid/parathyroid - C-cell hyperplasia, mild.
Spleen - inactive lymphoid component.
Lung - mild multifocal nonsuppurative perivascularitis.
Adrenal - cytoplasmic vacuolization, zona glomerulosa.
Mesenteric lymph node - erythrophagocytosis, active appearing node.
Prostate - minimal cystic dilatation of glands; mild epithelial vacuolization.
Pituitary - cystic Rathke's pouch remnant.
Brain - focal capillary ectasia.
Comment - all lesions considered to be incidental findings.

23 Feb 1978
Date

George A. Parker, D.V.M.
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>372</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>0 ppm</u>	PM NUMBER:	<u>77/11164</u>
DATE OF DEATH:	<u>12/7/77</u>	GROUP NUMBER:	<u>1.</u>
DEATH:	<u>Terminal kill</u>	SEX:	<u>Male</u>
METHOD OF KILL:	<u>OD Somlethol</u>	WEEK OF STUDY:	<u></u>
MORIBUND KILL:	<u></u>		

GROSS FINDINGS:

No gross lesion recognized.

MICROSCOPIC FINDINGS:

Thyroid/parathyroid - abundant C-cells; cystic ultimobranchial remnant, parathyroid.

Kidneys - microlithiasis, papilla.

Prostate - minimal cystic dilatation.

Brain - minimal focal neuronal swelling and degeneration, probably presection artefact.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.

PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>373</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>0 ppm</u>	PM NUMBER:	<u>77/11172</u>
DATE OF DEATH:	<u>12/8/77</u>		
DEATH:	<u>Terminal kill</u>	GROUP NUMBER:	<u>1</u>
METHOD OF KILL:	<u>OD Somilethol</u>	SEX:	<u>Male</u>
MORIBUND KILL:	<u></u>	WEEK OF STUDY:	<u></u>

GROSS FINDINGS:

No gross lesion recognized.

MICROSCOPIC FINDINGS:

Kidneys - microlithiasis, papilla.

Small intestine - focal mineralization, mucosa.

Testis - minimal giant cell degeneration and focal hypospermatogenesis.

Muscle - minimal focal chronic myositis.

Brain - occasional swollen axon, medulla oblongata.

23 Feb 1978
Date

George A. Parker, D.V.M.
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 374
DOSAGE: 0 ppm
DATE OF DEATH: 12/5/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL: _____

PROJECT NUMBER: 10734
PM NUMBER: 77/11149
GROUP NUMBER: 1
SEX: Female
WEEK OF STUDY: _____

GROSS FINDINGS:
No gross lesions recognized.

MICROSCOPIC FINDINGS:
Thyroid/parathyroid - mild C-cell hyperplasia.
Spleen - inactive lymphoid component.
Kidneys - mild nonsuppurative pyelitis.
Comment - all changes are common incidental findings.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>375</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>0 ppm</u>	PM NUMBER:	<u>77/11157</u>
DATE OF DEATH:	<u>12/6/77</u>	GROUP NUMBER:	<u>1</u>
DEATH:	<u>Terminal kill</u>	SEX:	<u>Female</u>
METHOD OF KILL:	<u>OD Somlethol</u>	WEEK OF STUDY:	<u></u>
MORIBUND KILL:	<u></u>		

GROSS FINDINGS:
No gross lesion recognized.

MICROSCOPIC FINDINGS:
No lesion recognized.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 376
DOSAGE: 0 ppm
DATE OF DEATH: 12/7/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL: _____

PROJECT NUMBER: 10734
PM NUMBER: 77/11165
GROUP NUMBER: 1
SEX: Female
WEEK OF STUDY: _____

GROSS FINDINGS:
No gross lesion recognized.

MICROSCOPIC FINDINGS:
Kidneys - microlithiasis, papilla.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 377
DOSAGE: 0 ppm
DATE OF DEATH: 12/8/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL: _____

PROJECT NUMBER: 10734
PM NUMBER: 77/11173
GROUP NUMBER: 1
SEX: Female
WEEK OF STUDY: _____

GROSS FINDINGS:
No gross lesion recognized.

MICROSCOPIC FINDINGS:
Thyroid/parathyroid - abundant C-cells.
Kidneys - microlithiasis, pelvis.
Urinary bladder - perivascular hyaline material, serosa; congestion.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>394</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>3000 ppm</u>	PM NUMBER:	<u>77/11154</u>
DATE OF DEATH:	<u>12/5/77</u>	GROUP NUMBER:	<u>4</u>
DEATH:	<u>Terminal kill</u>	SEX:	<u>Male</u>
METHOD OF KILL:	<u>OD Somlethol</u>	WEEK OF STUDY:	<u></u>
MORIBUND KILL:	<u></u>		

GROSS FINDINGS:

No gross lesion recognized.

MICROSCOPIC FINDINGS:

Spleen - very mild lymphoid depletion.
Lungs - substantial number of circulating neutrophils.
Liver - minimal vacuolar change.
Adrenal - vacuolization of zona glomerulosa cells.
Mesenteric lymph node - erythrophagocytosis.
Prostate - perinuclear vacuoles in a moderate number of prostatic epithelial cells.
Pituitary - cystic Rathke's pouch remnant.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 395
DOSAGE: 3000 ppm
DATE OF DEATH: 12/6/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL:

PROJECT NUMBER: 10734
PM NUMBER: 77/11162
GROUP NUMBER: 4
SEX: Male
WEEK OF STUDY:

GROSS FINDINGS:

No gross lesion recognized.

MICROSCOPIC FINDINGS:

Thyroid/parathyroid - cystic ultimobranchial remnant, thyroid.
Kidneys - mild multifocal mineralization, papilla.
Adrenal - vacuolization of zona glomerulosa.
Small intestine - cystic crypts.
Colon - mild multifocal mucosal congestion.
Pituitary - pronounced aggregation of pars intermedia cells. No expansion.

23 Feb 1978
Date

George A. Parker, D.V.M.
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>396</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>3000 ppm</u>	PM NUMBER:	<u>77/11170</u>
DATE OF DEATH:	<u>12/7/77</u>		
DEATH:	<u>Terminal kill</u>	GROUP NUMBER:	<u>4</u>
METHOD OF KILL:	<u>OD Somlethol</u>	SEX:	<u>Male</u>
MORIBUND KILL:	<u></u>	WEEK OF STUDY:	<u></u>

GROSS FINDINGS:

Heart - small (3mm) hard white nodule in aorta beneath one cusp of semilunar valve.
Kidneys - rim of cortex dark brown.

MICROSCOPIC FINDINGS:

Lungs - minimal multifocal perivascularitis; pleuritis; interstitial pneumonia.
Kidneys - mild mineralization, papilla.
Prostate - mild cystic glandular hyperplasia.
Heart - no lesion recognized.

23 Feb 1978
Date

George A. Parker, D.V.M.
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>397</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>3000 ppm</u>	PM NUMBER:	<u>77/11178</u>
DATE OF DEATH:	<u>12/8/77</u>	GROUP NUMBER:	<u>4</u>
DEATH:	<u>Terminal kill</u>	SEX:	<u>Male</u>
METHOD OF KILL:	<u>OD Somlethol</u>	WEEK OF STUDY:	<u></u>
MORIBUND KILL:	<u></u>		

GROSS FINDINGS:

No gross lesion recognized.

MICROSCOPIC FINDINGS:

Thyroid/parathyroid - cystic ultimobranchial remnants, parathyroid.
Liver - minimal vacuolar change.
Kidneys - microlithiasis, papilla. Several small (immature) glomeruli.
Small intestine - multifocal cystic crypts, mild.
Testis - mild multifocal hypospermatogenesis.
Urinary bladder - perivascular hyalinosis, serosa.

23 Feb 1978
Date

George A. Parker
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>398</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>3000 ppm</u>	PM NUMBER:	<u>77/11155</u>
DATE OF DEATH:	<u>12/5/77</u>		
DEATH:	<u>Terminal kill</u>	GROUP NUMBER:	<u>4</u>
METHOD OF KILL:	<u>OD Somlethol</u>	SEX:	<u>Female</u>
MORIBUND KILL:	<u></u>	WEEK OF STUDY:	<u></u>

GROSS FINDINGS:
No gross lesion recognized.

MICROSCOPIC FINDINGS:
Kidneys - mild mineralization, papilla.
Adrenal - mild vacuolization of zona glomerulosa cells.

23 Feb 1978
Date

George A. Parker, D.V.M.
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 399
DOSAGE: 3000 ppm
DATE OF DEATH: 12/6/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL: _____

PROJECT NUMBER: 10734
PM NUMBER: 77/11163
GROUP NUMBER: 4
SEX: Female
WEEK OF STUDY: _____

GROSS FINDINGS:
No gross lesion recognized.

MICROSCOPIC FINDINGS:

Ovary - several large corpora lutea.
Lungs - focal alveolar proteinosis; intra-alveolar hyaline bodies.
Kidneys - microlithiasis, papilla.
Uterus - secretory; no lesion recognized.
Urinary bladder - perivascular hyalinosis, serosa.
Mammary gland - active, not secretory, no lesion recognized.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER: 400
DOSAGE: 3000 ppm
DATE OF DEATH: 12/7/77
DEATH: Terminal kill
METHOD OF KILL: OD Somlethol
MORIBUND KILL: _____

PROJECT NUMBER: 10734
PM NUMBER: 77/11171
GROUP NUMBER: 4
SEX: Female
WEEK OF STUDY: _____

GROSS FINDINGS:

No gross lesion recognized.

MICROSCOPIC FINDINGS:

Thyroid/parathyroid - moderate nonsuppurative thyroiditis.
Ovary - has several large corpora lutea.
Lung - mineralith.
Liver - mottled due to glycogen in centrilobular areas.
Gallbladder - segments of epithelial cells have clear cytoplasm.
Kidneys - microliths, papilla; minimal multifocal glomerulosclerosis.
Uterus - secretory; no lesion recognized.
Urinary bladder - perivascular hyaline material, serosa.
Mammary gland - active, but not secretory.

23 Feb 1978
Date

George A. Parker, D.V.M.
Veterinary Pathologist

LITTON BIONETICS, INC.
PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>401</u>	PROJECT NUMBER:	<u>10734</u>
DOSAGE:	<u>3000 ppm</u>	PM NUMBER:	<u>77/11179</u>
DATE OF DEATH:	<u>12/8/77</u>	GROUP NUMBER:	<u>4</u>
DEATH:	<u>Terminal kill</u>	SEX:	<u>Female</u>
METHOD OF KILL:	<u>OD Somlethol</u>	WEEK OF STUDY:	<u></u>
MORIBUND KILL:	<u></u>		

GROSS FINDINGS:

Skin - two small (8mm) denuded areas in skin of left carpal and metacarpal areas.
Kidneys - very slight dark brownish coloration at periphery of cortex.

MICROSCOPIC FINDINGS:

Kidneys - minimal microlithiasis, papilla. Minimal focal nonsuppurative nephritis.
Skin - focal ulcerative dermatitis with scab formation. No obvious etiologic agent.

23 Feb 1978
Date

George A. Parker DVM
George A. Parker, D.V.M.
Veterinary Pathologist

THREE MONTH TOXICITY STUDY IN DOGS

LBI PROJECT NO. 10734-08

LEGEND FOR TABLE OF HISTOLOGIC FINDINGS

- + = Positive finding (ungraded lesion) encountered in a designated tissue or organ.
- = Negative finding in a designated tissue or organ; tissue examined.
- 1 = Positive finding graded "minimal".
- 2 = Positive finding graded "mild".
- 3 = Positive finding graded "moderate".

TABLE I-G-53

THREE-MONTH TOXICITY STUDY IN DOGS

LBI PROJECT NO. 10734-08

TABLE OF HISTOLOGIC FINDINGS

GROUP NUMBER	#1				#4				#4				#4			
	Males		Females		Males		Females		Males		Females		Males		Females	
SEX	370	371	372	373	374	375	376	377	394	395	396	397	398	399	400	401
ADRENAL GLAND																
SPINAL CORD																
THYROID/PARATHYROID	-	2	-	-	2	-	-	+	-	-	-	-	-	-	-	-
C-cell hyperplasia			+													
Abundant C-cells			+							+		+				
Cystic ultimobranchial remnant																
Non-suppurative thyroiditis																}
SCIATIC NERVE																
SPLIVER	-	+	-	-	+	-	-	-	2	-	-	-	-	-	-	-
Inactive lymphoid component																
Lymphoid depletion																
PANCREAS																
LUNG	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Intraalveolar hyaline bodies																
Emphysema																
Diffuse non-suppurative perivascularitis		2												+		+
Substantial number of circulating neutrophils									+							
Diffuse perivascularitis																
Interstitial pneumonia																
Pleuritis																
HEART																
LIVER	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Vacuolar change																
Glycogen in centrilobular areas																+

TABLE I-G-53 (Continued)

THREE-MONTH TOXICITY STUDY IN DOGS

LBI PROJECT NO. 10734-08

TABLE OF HISTOLOGIC FINDINGS

GROUP	NUMBER	#1				#4				#7							
		Males		Females		Males		Females		Males		Females					
SEX	ANIMAL NUMBER	370	371	372	373	374	375	376	377	394	395	396	397	398	399	400	401
GALLBLADDER																	
Clear cytoplasm, epithelial cells																	
KIDNEY																	
Microolithiasis																	
Non-suppurative pyelitis																	
Multifocal mineralization - papilla																	
Multifocal glomerulosclerosis																	
Non-suppurative nephritis																	
ADRENAL																	
Vacuolization																	
STOMACH																	
MESENTERIC LYMPH NODE																	
Active-appearing node																	
Erythrophagocytosis																	
SMALL INTESTINE																	
Cystic crypt																	
Focal mineralization, mucosa																	
COLON																	
Multifocal mucosal congestion																	
CECUM																	

TABLE I-G-53 (Continued)

THREE-MONTH TOXICITY STUDY IN DOGS

LBI PROJECT NO. 10734-08

TABLE OF HISTOLOGIC FINDINGS

GROUP NUMBER	#1				#2				#3				#4				#5																		
	SEX	ANIMAL NUMBER	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	
TESTIS																																			
Giant cell degeneration						1																													
Focal hypospermatogenesis						1																													
EPIDIDYMUS																																			
PROSTATE																																			
Cystic dilatation				1																															
Epithelial vacuolization				2																															
Cystic glandular hyperplasia																																			
MUSCLE																																			
Focal chronic myositis						1																													
URINARY BLADDER																																			
Perivascular hyaline material, serosa																																			
Constriction																																			
PANCREAS																																			
Active																																			
PITUITARY																																			
Cystic Rathke's pouch remnant																																			
Pronounced aggregation of pars intermedia cells																																			
RIB JUNCTION																																			

TABLE I-G-53 (Continued)

THREE-MONTH TOXICITY STUDY IN DOGS

LB1 PROJECT NO. 10734-08

TABLE OF HISTOLOGIC FINDINGS

GROUP NUMBER	#1				#4				#74							
	Males		Females		Males		Females		Males		Females					
ANIMAL NUMBER	370	371	372	373	374	375	376	377	394	395	396	397	398	399	400	401
STERNUM	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BRAIN	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
focal capillary ectasia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
focal neuronal swelling	-	-	1	+	-	-	-	-	-	-	-	-	-	-	-	-
Swollen axon medulla oblongata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
degeneration	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
EYE	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SKIN	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
dermatitis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
scabs	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OVARIES	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
large corpora lutea	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
UTERUS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
secretory	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

TABLE I-G-54
THREE MONTH TOXICITY STUDY IN DOGS
LBI PROJECT NO. 10734-08

INCIDENCE TABLE

GROUP NUMBER SEX	1	1	1	4	4	4	4
NUMBER OF ANIMALS EXAMINED	Males	Females	Males	Females	Males	Females	Males
	4	4	4	4	4	4	4
<u>THYROID/PARATHYROID</u>							
C-cell hyperplasia	1	1			0		0
Abundant C-cells	1	1			0		0
Cystic ultimobranchial remnant	1	0			2		0
Non-suppurative thyroiditis	0	0			0		1
<u>SPLEEN</u>							
Inactive lymphoid component	1	1			0		0
Lymphoid depletion	0	0			1		0
<u>LUNG</u>							
Intraalveolar hyaline bodies	0	0			0		1
Mineralith	0	0			0		1
Multifocal non-suppurative perivascularitis	1	0			0		0
Substantial number of circulating neutrophils	0	0			1		0
Multifocal perivascularitis	0	0			1		0
Interstitial pneumonia	0	0			1		0
Pleuritis	0	0			1		0
<u>LIVER</u>							
Vacuolar change	0	0			2		0
Glycogen in centrilobular areas	0	0			0		1
<u>GALLBLADDER</u>							
Clear cytoplasm, epithelial cells	0	0			0		1

TABLE I-G-54 (Continued)

LBI PROJECT NO. 10734-08
INCIDENCE TABLE (Continued)

GROUP NUMBER	1	4	1	4	4	4
SEX						
NUMBER OF ANIMALS EXAMINED	Males	Females	Males	Females	Males	Females
	4	4	4	4	4	4
<u>KIDNEYS</u>						
<u>Microolithiasis</u>	2	2	1	3		
Non-suppurative pyelitis	0	1	0	0		
Multifocal mineralization	0	0	2	1		
Multifocal glomerulosclerosis	0	0	0	1		
Non-suppurative nephritis	0	0	0	1		
<u>ADRENALS</u>						
<u>Vacuolization</u>	1	0	2	1		
<u>MESENTERIC LYMPH NODES</u>						
Active appearing node	2	0	0	0		
Erythrophagocytosis	1	0	1	0		
<u>SMALL INTESTINE</u>						
Cystic crypt	1	0	2	0		
Focal mineralization	1	0	0	0		
<u>COLON</u>						
Multifocal mucosal congestion	0	0	1	0		
<u>TESTIS</u>						
Giant cell degeneration	1	0	0	0		
<u>PROSTATE</u>						
Cystic dilatation	2	0	0	0		
Epithelial vacuolization	1	1	1	1		
Cystic glandular hyperplasia	0	1	1	1		

TABLE I-G-54 (Continued)

LBI PROJECT NO. 10734-08
INCIDENCE TABLE (Continued)

GROUP NUMBER SEX NUMBER OF ANIMALS EXAMINED	Males 1 4	Females 1 4	Males 4 4	Females 4 4
<u>MUSCLE</u> Focal chronic myositis	1	0	0	0
<u>URINARY BLADDER</u> Perivascular hyaline material Congestion	0 0	1 1	1 0	2 0
<u>MAMMARY GLAND</u> Active	0	0	0	2
<u>PITUITARY</u> Cystic Rathke's pouch remnant Pronounced aggregation of pars intermedia cells	1 0	0 0	1 1	0 0
<u>BRAIN</u> Focal capillary ectasia Focal neuronal swelling Swollen axon, medulla oblongata Degeneration	1 1 1 1	0 0 0 0	0 0 0 0	0 0 0 0
<u>SKIN</u> Dermatitis Scabs	0 0	0 0	0 0	1 1
<u>OVARIES</u> Large corpora lutea		0		2
<u>UTERUS</u> Secretory		0		2

PART II - SECTION A
INTRODUCTION AND MATERIAL

DCPD

In a continuation of an evaluation of the mammalian toxicity of DCPD, this compound has been studied by subchronic (90 day) administration to dogs, for reproductive and teratologic effects in rats, for mutagenic effects in certain tester strains of salmonella and additional aspects of its metabolic fate.

Earlier parts of the evaluation were reported in November 1976 under Contract No. DAMD 17-75-C-5068.

2. MATERIAL

DCPD (Dicyclopentadiene) also known as 3a,4,7,7a-Tetrahydro-4,7-methanoindene, was purchased from MC/B, 2909 Highland Avenue, Norwood, Ohio 45212, under catalog number TX 310. A single batch of 650 g was received on August 18, 1976, and assigned LBI No. 763A.

Analysis of DCPD was performed with a UC-W98 column. The retention time of the compound was 1.9 minutes. Trace impurities were noted at approximately 1.5 minutes and 2.1 minutes. The purity of DCPD appeared to be 98 to 99%, which is consistent with the MC/B assay of 99.79%. It cannot now be determined if one of these impurities may be the cis form.

Because of poor water solubility, DCPD was prepared for administration to animals by dissolving it in corn oil (Mazola) at concentrations appropriate to the various studies. The handling of DCPD itself was facilitated by slight warming, which converted the waxy solid to an easily measured liquid.

PART II - SECTION B
MICROBIAL MUTAGENESIS

DCPD

LBI PROJECT NO. 10734-01

SUMMARY

The test compound, DCPD Lot No. 040667, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

The test compound, DCPD W-761226, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: January 17, 1977
2. Description: Colorless liquid; DCPD Lot No. 040667

B. Indicator Microorganisms

Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (diabasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^aConcentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

TABLE II-B-1

LITTON HIONETICS, INC.

4. SUMMARY OF PLATE TEST RESULTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: DCPD LOT #040667

B. SOLVENT: DMSO

C. TEST DATE: FEB. 7, 1977

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	ISSUE	B-E-V-E-B-I-A-N-I-S-P-E-B-P-L-A-I-E									
			1A-1535	1A-1537	1A-1538	1A-98	1A-100	1A-100	1A-100	1A-100	1A-100	1A-100
NONACTIVATION	---	---	1	2	1	2	1	2	1	2	1	2
	SOLVENT CONTROL	---	20	15	14	24	142	182	142	182	142	182
	POSITIVE CONTROL	---	>1000	1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	TEST COMPOUND	0.00100 UL	13	6	16	25	147	147	147	147	147	147
		0.01000 UL	15	13	19	32	133	133	133	133	133	133
		0.10000 UL	10	15	24	34	156	156	156	156	156	156
ACTIVATION	---	---	8	10	15	15	159	159	159	159	159	159
		1.00000 UL	0	6	4	8	76	76	76	76	76	76
		5.00000 UL	0	6	4	8	76	76	76	76	76	76
		10.00000 UL	0	6	4	8	76	76	76	76	76	76
ACTIVATION	---	---	42	21	26	35	47	194	47	194	47	194
	SOLVENT CONTROL	---	>1000	297	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	POSITIVE CONTROL	---	>1000	297	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
	TEST COMPOUND	0.00100 UL	45	17	19	42	182	182	182	182	182	182
		0.01000 UL	38	24	13	47	191	191	191	191	191	191
		0.10000 UL	34	15	18	26	181	181	181	181	181	181
ACTIVATION	---	---	26	15	11	23	186	186	186	186	186	186
		1.00000 UL	23	17	2	132	15	15	15	15	15	15
		5.00000 UL	23	17	2	132	15	15	15	15	15	15
		10.00000 UL	23	17	2	132	15	15	15	15	15	15

* IBY* CONVERTANTS PER PLATE

** 1A-1535	HNMG	10 UG/PLATE	** 1A-1535	ANTH	100 UG/PLATE
1A-1537	QH	10 UG/PLATE	1A-1537	ANQ	100 UG/PLATE
1A-1538	NF	100 UG/PLATE	1A-1538	AAF	100 UG/PLATE
1A-98	NF	100 UG/PLATE	1A-98	AAF	100 UG/PLATE
1A-100	HNMG	10 UG/PLATE	1A-100	ANTH	100 UG/PLATE
04	HNMG	10 UG/PLATE	04	DMNA	100 MICROMOLES/PLATE
SOLVENT	DMSO	2.5 %/PLATE	SOLVENT	DMSO	2.5 %/PLATE

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 μ l to 5 μ l per plate. The compound was toxic to the strains TA-1535, TA-1537, TA-1538, TA-98, and the yeast strain D4 at 5 μ l per plate in the nonactivation assays.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

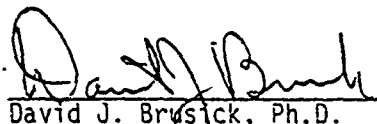
C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions


The test compound, DCPD Lot No. 040667, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:


David J. Brusick, Ph.D.
Director
Department of Genetics

2/27/77
Date

Reviewed by:


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Vice President

2/27/77
Date

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a

6. EVALUATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

STANDARD OPERATING PROCEDURES

To ensure an accurate and reliable mutagenicity testing program, LBI instituted the following procedures:

- The test compound was registered in a bound log book recording the date of receipt, complete client identification, physical description and LBI code number.
- Complete records of weights and dilutions associated with the testing of the submitted material were entered into a bound notebook.
- Raw data information was recorded on special printed forms that were dated and initialed by the individual performing the data collection at the time the observations were made. These forms were filed as permanent records.
- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

1. Date Received: January 17, 1977
2. Description: Colorless liquid; DCPD W-761226

B. Indicator Microorganisms

- Salmonella typhimurium, strains: TA-1535 TA-98
TA-1537 TA-100
TA-1538

Saccharomyces cerevisiae, strain: D4

C. Activation System (Ames et al., Mutation Research 31:347, 1975)

1. Reaction Mixture

<u>Component</u>	<u>Final Concentration/ml</u>
TPN	4 μ moles
Glucose-6-phosphate	5 μ moles
Sodium phosphate (diabasic)	100 μ moles
MgCl ₂	8 μ moles
KCl	33 μ moles
Homogenate fraction equivalent to 25 mg of wet tissue	0.1-0.15 ml 9,000 x g supernatant of rat liver

2. S-9 Homogenate

A 9,000 x g supernatant was prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 five days prior to kill.

2. MATERIALS (Continued)

D. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL</u> ^a	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS ^b
	2-Nitrofluorene (NF)	Dimethylsulfoxide ^c	FS ^b
	Quinacrine mustard (QM)	Water or saline	FS ^b
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide ^c	BPS ^b
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide ^c	FS ^b
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide ^c	FS ^b

^aConcentrations given in Results Section

^bBPS = Base-pair substitution

FS = Frameshift

^cPreviously shown to be nonmutagenic

E. Solvent

Either deionized water or dimethylsulfoxide (DMSO) was used to prepare stock solutions of solid materials. All dilutions of test materials were made in either deionized water or DMSO. The solvent employed and its concentration are recorded in the Results Section.

3. EXPERIMENTAL DESIGN

A. Plate Test (Overlay Method*)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to separate test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, at least four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests, a minimum of four different concentrations of the test chemical were added to the appropriate tubes with cells. Just prior to pouring, an aliquot of reaction mixture (0.5 ml containing the 9,000 x g liver homogenate) was added to each of the activation overlay tubes, which were then mixed, and the contents poured over the surface of a minimal agar plate and allowed to solidify. The plates were incubated for 48 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

B. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were analyzed in a computer program and reported on a printout. The results are presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points. Other relevant data are provided on the computer printout.

*Certain classes of chemicals known to be mutagens and carcinogens do not produce detectable responses using the standard Ames overlay method. Some dialkyl nitrosamines and certain substituted hydrazines are mutagenic in suspension assays, but not in the plate assay. Chemicals of these classes should be screened in a suspension assay.

TABLE II-B-2

A. SUMMARY OF PLATE TEST RESULTS

LITTON BIOMETRICS, INC.

NAME OR CODE DESIGNATION OF THE TEST COMPOUND: DCPDW-761226

SOLVENT: DMSO

TEST DATE: FEB. 7, 1977

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

TEST	SPECIES	ISSUE	B E V E Q I A N I S P E B P L A I F									
			1A-1535		1A-1537		1A-1538		1A-98		1A-100	
			1	2	1	2	1	2	1	2	1	2
NONACTIVATION												
SOLVENT CONTROL	---	---	20	15	14	14	24	182	12			
POSITIVE CONTROL**	---	---	>1000	>1000	>1000	>1000	>1000	>1000	262			
TEST COMPOUND												
0.00100 UL	---	---	14	10	14	14	87	183	15			
0.01000 UL	---	---	12	16	12	12	25	140	4			
0.10000 UL	---	---	9	14	18	14	26	145	8			
1.00000 UL	---	---	9	19	5	5	14	133	4			
5.00000 UL	---	---	0	5	4	4	0	43	1			
ACTIVATION												
SOLVENT CONTROL	RAT	LIVER	42	23	26	26	22	201	47			
POSITIVE CONTROL***	RAT	LIVER	>1000	297	>1000	>1000	>1000	>1000	398			
TEST COMPOUND												
0.00100 UL	RAT	LIVER	47	13	18	18	30	169	43			
0.01000 UL	RAT	LIVER	37	26	20	20	24	159	56			
0.10000 UL	RAT	LIVER	41	20	13	13	25	159	56			
1.00000 UL	RAT	LIVER	23	23	5	5	26	276	84			
5.00000 UL	RAT	LIVER	16	14	0	0	4	35	89			
10.00000 UL	RAT	LIVER	-	-	-	-	-	-	21			

* ILY+ CONVENTANTS PER PLATE

**	1A-1535	HNNG	10 UG/PLATE	***	1A-1535	ANTH	100 UG/PLATE
	1A-1537	QH	10 UG/PLATE		1A-1537	AMQ	100 UG/PLATE
	1A-1538	NF	100 UG/PLATE		1A-1538	AAF	100 UG/PLATE
	1A-98	NF	100 UG/PLATE		1A-98	AAF	100 UG/PLATE
	1A-100	HNNG	10 UG/PLATE		1A-100	ANTH	100 UG/PLATE
	D4	HNNG	10 UG/PLATE		D4	DMNA	100 MICROMOLES/PLATE
	SOLVENT	DMSO	2.5 %/PLATE		SOLVENT	DMSO	2.5 %/PLATE

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested over a series of concentrations such that there was either quantitative or qualitative evidence of some chemically-induced physiological effects at the high dose level. The low dose in all cases was below a concentration that demonstrated any toxic effect. The dose range employed for the evaluation of this compound was from 0.001 μ l to 5 μ l per plate. The compound was toxic to all the strains at 5 μ l per plate.

B. Nonactivation Test Results

The results of the tests conducted on the compound in the absence of a metabolic system were all negative.

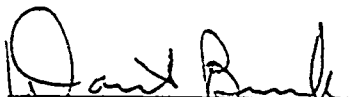
C. Activation Test Results

The results of the tests conducted on the compound in the presence of the rat liver activation system were all negative.

D. Conclusions

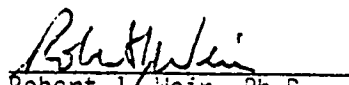
The test compound, DCPD W-761226, did not demonstrate mutagenic activity in any of the assays conducted in this evaluation and was considered not mutagenic under these test conditions.

Submitted by:


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Director
Department of Genetics

2/24/77
Date

Reviewed by:


Robert J. Weir, Ph.D.
Vice President

2/25/77
Date

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually, mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

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If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control value.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a

6. EVALUATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

D. Evaluation Criteria for Ames Assay

3. Pattern

given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

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If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

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E. Relationship Between Mutagenicity and Carcinogenicity

It must be emphasized that the Ames Salmonella/microsome test is not a definitive test for chemical carcinogens. It is recognized, however, that correlative and functional relationships have been demonstrated between these two end points. The results of comparative tests on 300 chemicals by McCann et al. (Proc. Nat. Acad. Sci. USA, 72:5135-5139, 1975) show an extremely good correlation between results of microbial mutagenesis tests and in vivo rodent carcinogenesis assays.

All evaluation and interpretation of the data presented in this report are based only on the demonstration of or lack of mutagenic activity.

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- All animal tissue S-9 preparations used in the activation tests were taken from dated and pretested frozen lots identified by a unique number. The S-9 preparations were monitored for uniformity and the information recorded.

PART II - SECTION C
PHARMACOKINETICS AND METABOLISM

DCPD

LBI PROJECT NO. 10734-02

SUMMARY

With the concurrence of the Project Officer, emphasis was placed on DIMP rather than DCPD with the result that no additional significant information was developed regarding DCPD. Previous findings are summarized below:

DCPD was absorbed after oral administration to mice, rats, and dogs. Peak plasma levels occurred in 2 hours in mice and dogs, and in 6 hours in rats. DCPD was widely distributed in all three species at 1 to 2 hours with the highest levels in urinary bladder, gall bladder and body fat in mice, in gall bladder and bile in dogs, and in body fat, adrenals and urinary bladder in rats. Excretion appeared to be primarily via the urine in all three species. About 85% of the administered radioactivity was accounted for in urine and feces within 24 hours. Urine from mice and dogs showed two radioactive components while rat urine also contained a third. All of these seemed to differ from DCPD on TLC, but none has yet been identified.

PART II - SECTION D

TERATOLOGY IN RATS

DCPD

LBI PROJECT NO. 10734-05

SUMMARY

The test material was administered in the diet at doses of 80, 250 and 750 ppm to pregnant female rats on Days 6 through 15 of gestation. There were no changes in the dams or among the fetuses that indicated an adverse compound-related effect.

1. OBJECTIVE

The objective of this study was to investigate the effect of the test material on fetuses during the period of organogenesis when administered to the pregnant rat.

2. MATERIAL

Refer to Part II - Section A.

3. EXPERIMENTAL DESIGN

Female [CRL:COBS CD (SD) BR] rats were obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for 12 days. At that time, each female was paired with a sexually mature male of the same strain and from the same supplier. The females were examined daily for the presence of a copulatory plug. The presence of such a plug was taken as evidence of mating and designated as Day 0 of gestation. The female rats were 11 weeks of age at the time of the first dose (June 13, 1977). Mated female rats were assigned sequentially to treatment groups and identified by cage cards as indicated below.

<u>Group Number</u>	<u>Female Rat Numbers</u>	<u>Dose (ppm)</u>
1	6615-6635	0 (Control)
2	6640-6659	80
3	6665-6684	250
4	6690-6709	750

3. EXPERIMENTAL DESIGN (Continued)

The female rats were individually housed in wire cages in a temperature-controlled animal room with artificial illumination automatically controlled to provide a 12 hour light cycle. No other species were housed in this animal room during the course of the study. No other test materials were under concurrent investigation in this animal room. The appropriate diets and fresh water (acidified pH 2.5) were provided ad libitum.

The test material was incorporated into the basal diet (Purina Laboratory Chow) on gestation Days 6 through 15 so as to provide the dose levels indicated previously. The test material (0.8, 2.5 or 7.5 g) was suspended in 300 ml of corn oil and blended with 10 kg of the basal diet in a twin shell blender for 15 minutes. The control diet contained 300 ml of corn oil per 10 kg of meal. The dose levels used in this study were approved by Dr. E. Ross Hart of LBI based on previous studies.

Although the revised protocol for this study indicated that the female rats be killed on Day 20 of gestation, the original protocol which called for termination on Day 19 of gestation was inadvertently followed. This was not judged to affect the integrity of the study.

The mated female rats were weighed on Days 0, 6, 16 and 19 of gestation. Food consumption was measured during the period 0-6, 6-16 and 16-19 days of gestation. The female rats were observed daily for changes in general appearance, behavior and condition.

On Day 19 of gestation, the adult female rats were anesthetized with chloroform, and the visceral and thoracic organs examined. The uterus was removed and opened. The number of implantation sites and their placement in the uterine horns, live and dead fetuses, and resorption sites were recorded. The fetuses were removed, examined externally for abnormalities and weighed.

One third of the fetuses of each litter were fixed in Bouin's fluid. These were later examined for changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses of each litter were examined for skeletal abnormalities following staining with Alizarin Red S.

The uterus and ovaries from the adult females were preserved in 10% formalin for possible future examination. No further examination was judged to be necessary.

3. EXPERIMENTAL DESIGN (Continued)

Statistical analysis of the data was performed using the litter as a basic sampling unit. This concept has been widely supported with regard to teratology [1,2]. Dunnett's t-test [3] was used to determine statistical significance ($p < 0.05$) with regard to difference between means with near normal distribution (body weights and food consumption of dams, mean pup weight based on litter averages). Ratios, for example sex ratio and pregnancy ratio, were analyzed with a 2x2 contingency table with Yates' correction [4]. With regard to discontinuous parameters as measured by the number of abnormal fetuses within a litter, Wilcoxon Rank Sum [5] was used.

4. RESULTS

No deaths occurred among the adult female rats, and except for Group 2 female rat no. 6654, these animals were normal in appearance throughout the study. Female rat no. 6654 was emaciated and had an arched back and a red crust around the nose and mouth on Day 19 of gestation.

Examination of the females at necropsy revealed a dark red area in the lungs of female rat no. 6650 (Group 2) and a liver mottled with small white spots in female no. 6698 (Group 4). These changes were not considered to be related to dose.

Mean body weight and food consumption, as shown in the Appendix Table 1, indicated no significant difference between control and treated pregnant rats [3].

Based on the observations of the uterine contents obtained on Day 19 of gestation, the test material did not produce any effect. These data have been summarized in Text Table A, and the details have been tabulated in Table 2 included in the Appendix.

Examination of the offspring at delivery revealed subcutaneous hematomas in fetuses from litters at all dose levels as tabulated below.

<u>Dose (ppm)</u>	<u>Number of Fetuses with Subcutaneous Hematomas (litters)</u>
0 (Control)	41 (10)
80	15 (7)
250	29 (11)
750	34 (14)

LITTON BIONETICS, INC.
PROJECT NO. 734-05

TABLE II-D-3

TABLE A

SUMMARY OF REPRODUCTIVE PERFORMANCE

	DOSE (PPM)			STATISTICAL METHOD
	0	80	250	750
PREGNANCY RATIO (PREGNANT/BRED)	19/21	20/20	19/20	19/20
LIVE LITTERS	19/19 (100%)	20/20 (100%)	19/19 (100%)	19/19 (100%)
				[4]
IMPLANTATION SITES (LEFT HORN/RIGHT HORN)	154/159	132/168	132/160	134/158
				[4]
RESORPTIONS	18	22	19	13
				[5]
LITTERS WITH RESORPTIONS	14 (74%)	8 (40%)	11 (58%)	8 (42%)
				[4]
DEAD FETUSES	0	0	0	0
				[5]
LITTERS WITH DEAD FETUSES	0	0	0	0
				[4]
LIVE FETUSES/IMPLANTATION SITE	295/313 (94%)	278/300 (93%)	273/292 (93%)	279/292 (96%)
				[4]
MEAN LIVE LITTER SIZE (FETUSES)	15.5	13.9	14.4	14.7
				[3]
AVERAGE FETAL WEIGHT (G)	2.3	2.3	2.4	2.4
				[3]
AVERAGE FETAL LENGTH (CM)	2.7	2.6	2.7	2.7
				[3]

4. RESULTS (Continued)

Although there was a significant decrease in the number of fetuses with subcutaneous hematomas in Group 2 [4], this was not judged to be a dose-related response. Other observations on fetuses at delivery included one fetus of female no. 6622 (Group 1) with swelling of the right hind limb and one fetus of female no. 6617 (Group 1) with intestines protruding at the umbilicus.

Examination of the Bouin's fixed specimens revealed (in addition to the previously mentioned protruding intestines in the fetus of litter no. 6617) the absence of the left kidney in one fetus of litter no. 6620 (Group 1), enlarged kidneys in one fetus of litter no. 6645 (Group 2), and unilateral anophthalmia in one fetus of litter no. 6709 (Group 4). These changes did not indicate a dose-related response. The sex and number of fetuses examined for soft tissue changes were as follows.

<u>Dose (ppm)</u>	<u>Males</u>	<u>Females</u>
0 (Control)	48	46
80	40	46
250	47	39
750	40	47

The sex ratio did not differ significantly between treated and control groups [4].

The results of the skeletal examination of the cleared and stained fetuses have been detailed in Appendix Table 3. Most of the changes noted, while not strictly normal, are frequently observed in 19 day old rat fetuses of this strain and source in our laboratory. These changes have been summarized below.

<u>Dose (ppm)</u>	<u>Number Fetuses Examined</u>	<u>Number Fetuses Normal</u>	<u>Fetuses With Commonly Encountered Changes Only</u>	<u>Fetuses With Unusual Skeletal Variations</u>
0 (Control)	199 ^a (19) ^b	106	91 (17)	2 (2)
80	192 (20)	85	103 (19)	4 (3)
250	187 (19)	92	95 (13)	0 (0)
750	192 (19)	91	98 (17)	3 (2)

^aTwo specimens of litter no. 6634 lost during processing and handling, not examined.

^bNumber of litters in parentheses.

4. RESULTS (Continued)

The unusual changes for the most part were related to retarded bone ossification and were not malformations as such. Neither the frequency nor the character of these changes indicated an adverse effect on fetal growth and development, or a teratogenic potential [4,5].

Thirty days after transmittal of this report, original data from the Department of Toxicology will be transferred to the LBI Archives, 5516 Nicholson Lane, Kensington, Maryland. A copy of this report was reviewed by the LBI Quality Assurance Unit.

5. CONCLUSION

Administration of the test material to female rats by incorporation into the diet at 80, 250 and 750 ppm produced no effect on the pregnant dams. There was no evidence of compound-induced terata, variation in sex ratio, embryo toxicity or inhibition of fetal growth and development.

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LITTON BIONETICS, INC.
PROJECT NO. 10734-05

APPENDIX

TABLE 1 BODY WEIGHTS AND FOOD CONSUMPTION OF PREGNANT RATS

TABLE 2 OBSERVATIONS AT CAESAREAN SECTION

TABLE 3 OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE II-D-4

TABLE 1

BODY WEIGHTS AND FOOD CONSUMPTION OF PREGNANT RATS

DOSE (PPM).		MEAN BODY WEIGHTS IN GRAMS ^a				MEAN DAILY FOOD CONSUMPTION IN GRAMS ^a			
		DAY 0	DAY 6	DAY 15	DAY 19	DAY 0-6	DAY 6-15	DAY 16-19	
0 (CONTROL)	MEAN	218	244	296	350	19	20	26	
	SD	13	13	18	20	4	4	3	
	SE	3.1	3.0	4.1	4.7	1.0	1.1	0.8	
	N	19	19	19	19	17	11	19	
80	MEAN	214	244	289	334	20	19	23	
	SD	18	13	34	45	4	3	5	
	SE	4.1	2.9	7.5	10.0	1.0	0.8	1.2	
	N	20	20	20	20	15	18	20	
250	MEAN	223	247	295	346	22	20	26	
	SD	12	18	15	17	5	1	4	
	SE	2.8	4.2	3.4	3.8	1.2	0.3	0.9	
	N	19	19	19	19	15	11	18	
750	MEAN	213	243	291	342	22	20	26	
	SD	18	14	19	27	4	1	3	
	SE	4.1	3.1	4.4	6.3	1.0	0.3	0.6	
	N	19	19	19	19	16	11	19	

^a Calculations do not include non-pregnant females.

TABLE II-D-5

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 2

OBSERVATIONS AT CAESAREAN SECTION

DOSE - 0 PPM

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES	
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (G)
6615	7	8	1	0	14	2.9
6616	6	11	1	0	16	2.8
6617	11	6	0	0	17	1.8
6618	0	0	0	0	0	---
6619	9	8	1	0	16	2.5
6620	6	8	0	0	14	2.5
6621	7	10	0	0	17	2.2
6622	10	5	0	0	15	2.2
6623	9	10	1	0	18	2.4
6624	7	8	1	0	14	2.2
6625	9	8	2	0	15	2.4
6626	0	0	0	0	0	---
6627	6	7	1	0	12	2.3
6628	10	6	1	0	15	2.4
6629	10	10	1	0	19	2.2
6630	4	12	1	0	15	2.2
6631	5	7	0	0	12	2.2
6632	9	11	1	0	19	2.1
6633	8	6	1	0	13	2.0
6634	12	6	1	0	17	1.8
6635	9	12	4	0	17	1.9
TOTAL	154	159	18	0	295	
MEAN*					15.5	2.3
SD					2.09	0.29
SE					0.480	0.067
N	19	19	19	19	19	19

*Mean, SD and SE do not include non-pregnant females.

TABLE II-D-5 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 2 (CONTINUED)

OBSERVATIONS AT CAESAREAN SECTION

DOSE - 80 PPM

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIALE FETUSES	
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (G)
6640	3	10	9	0	4	3.0
6641	8	11	0	0	19	2.3
6642	0	6	1	0	5	3.0
6643	7	8	0	0	15	2.7
6644	9	8	0	0	17	2.7
6645	6	13	0	0	19	2.4
6646	7	7	0	0	14	1.9
6647	8	10	0	0	18	2.0
6648	3	12	5	0	10	2.5
6649	9	1	1	0	9	1.7
6650	7	9	0	0	16	2.3
6651	7	8	1	0	14	2.2
6652	9	7	0	0	16	2.7
6653	8	7	0	0	15	2.2
6654	7	7	1	0	13	1.6
6655	7	7	0	0	14	2.4
6656	6	12	2	0	16	2.1
6657	10	6	0	0	16	2.1
6658	6	9	0	0	15	2.7
6659	5	10	2	0	13	1.9
TOTAL	132	168	22	0	278	
MEAN					13.9	2.3
SD					4.09	0.40
SE					0.915	0.089
N	20	20	20	20	20	20
						2.6
						0.25
						0.057
						2.4

TABLE II-D-5 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 2 (CONTINUED)

OBSERVATIONS AT CAESAREAN SECTION

DOSE - 250 PPM

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES		
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (G)	MEAN LENGTH (CM)
6665	9	5	0	0	14	2.9	3.1
6666	9	9	2	0	16	2.9	3.1
6667	8	4	1	0	11	2.0	2.3
6668	7	7	0	0	14	2.8	3.0
6669	6	9	0	0	15	2.6	2.9
6670	7	7	0	0	14	2.7	2.9
6671	5	10	2	0	13	2.1	2.3
6672	5	9	3	0	11	2.7	2.9
6673	6	10	0	0	16	2.4	2.7
6674	0	0	0	0	0	----	----
6675	6	12	2	0	16	2.1	2.4
6676	8	10	2	0	16	2.5	2.7
6677	4	12	2	0	14	2.2	2.5
6678	5	8	1	0	12	2.2	2.5
6679	5	5	2	0	8	2.7	3.1
6680	9	9	0	0	18	2.3	2.6
6681	11	6	0	0	17	1.8	2.3
6682	8	7	0	0	15	2.1	2.6
6683	8	10	1	0	17	2.0	2.3
6684	6	11	1	0	16	3.5	3.4
TOTAL	132	160	19	0	273		
MEAN*					14.4	2.4	2.7
SD					2.50	0.42	0.33
SE					0.573	0.097	0.077
N	19	19	19	19	19	19	19

*Mean, SD and SE do not include non-pregnant female.

TABLE II-D-5 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 2 (CONTINUED)

OBSERVATIONS AT CAESAREAN SECTION

DOSE - 750 PPM

FEMALE NUMBER	IMPLANTATION SITES		RESORPTION SITES	DEAD FETUSES	VIABLE FETUSES		
	LEFT HORN	RIGHT HORN			NUMBER	MEAN WEIGHT (G)	MEAN LENGTH (CM)
6690	8	7	1	0	14	2.6	2.9
6691	6	13	0	0	19	2.5	2.8
6692	6	8	2	0	12	2.9	3.1
6693	9	11	2	0	18	2.6	2.8
6694	4	8	0	0	12	3.1	3.1
6695	7	7	0	0	14	2.8	2.9
6696	5	11	0	0	16	2.6	2.8
6697	11	5	0	0	16	3.0	3.0
6698	5	11	0	0	16	2.5	2.7
6699	6	8	0	0	14	2.5	2.7
6700	6	9	0	0	15	1.7	2.2
6701	10	4	2	0	12	2.2	2.3
6702	11	5	3	0	13	2.0	2.1
6703	4	12	1	0	15	2.3	2.6
6704	0	0	0	0	0	----	----
6705	7	13	0	0	20	1.9	2.5
6706	8	11	0	0	19	2.1	2.7
6707	5	0	0	0	5	2.2	2.7
6708	5	7	1	0	11	1.6	2.1
6709	11	8	1	0	18	2.2	2.5
TOTAL	134	158	13	0	279		
MEAN*					14.7	2.4	2.7
SD					3.54	0.42	0.31
SE					0.813	0.096	0.071
N	19	19	19	19	19	19	19

*Mean, SD and SE do not include non-pregnant female.

TABLE II-D-6

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 0 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6615	10	8 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE. * 1 STERNEBRAE MALALIGNED, NON-FUSED OSSIFICATION CENTERS OF THE STERNEBRAE.
6616	10	8 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSI- FIED.
6617	12	9 REDUCED OSSIFICATION OF THE HYOID BONE, STERNE- BRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE ISCHIUM, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED.
6619	11	7 NO VISIBLE ABNORMALITIES. 2 UNILATERAL RIB 14. 2 REDUCED OSSIFICATION OF THE PUBES.
6620	10	9 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES.
6621	12	11 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED.

*Some of these findings not commonly encountered.

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 0 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6622	10	2 NO VISIBLE ABNORMALITIES. 8 REDUCED OSSIFICATION OF THE PUBES.
6623	12	10 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE PUBES.
6624	9	5 NO VISIBLE ABNORMALITIES. 3 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED.
6625	10	10 NO VISIBLE ABNORMALITIES.
6627	8	8 NO VISIBLE ABNORMALITIES.
6628	10	9 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE PUBES.
6629	13	4 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 3 PUBES NOT OSSIFIED. 2 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, NON-FUSED OSSIFICATION CENTERS OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, NON-FUSED OSSIFICATION CENTERS OF THE STERNEBRAE, (PORTIONS OF FORE EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).
6630	10	7 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 0 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6631	8	<p>5 NO VISIBLE ABNORMALITIES.</p> <p>1 PUBES NOT OSSIFIED.</p> <p>1 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED.</p> <p>1 REDUCED OSSIFICATION OF THE HYOID BONE, STERNEBRAE NOT OSSIFIED.</p>
6632	13	<p>3 REDUCED OSSIFICATION OF THE STERNEBRAE, (PORTIONS OF SKULL, PELVIC GIRDLE AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).</p> <p>1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES, (PORTIONS OF THE SKULL AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).</p> <p>1 PUBES NOT OSSIFIED, (PORTIONS OF THE SKULL AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).</p> <p>1 UNILATERAL RIB 14, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, (PORTIONS OF SKULL, PELVIC GIRDLE AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).</p> <p>1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, REDUCED OSSIFICATION OF THE PUBES.</p> <p>1 PUBES NOT OSSIFIED, (PORTIONS OF SKULL AND FORE EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).</p> <p>1 REDUCED OSSIFICATION OF THE PUBES, (PORTIONS OF THE SKULL AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).</p>

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 0 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6632 (CONTD)	13	<ul style="list-style-type: none"> 1 REDUCED OSSIFICATION OF THE STERNEBRAE, UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES, (PORTIONS OF FORE EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY). 1 REDUCED OSSIFICATION OF THE STERNEBRAE, UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED, (PORTIONS OF SKULL DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY). 1 REDUCED OSSIFICATION OF THE STERNEBRAE, (PORTIONS OF THE SKULL, PELVIC GIRDLE, FORE AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY). 1 REDUCED OSSIFICATION OF THE STERNEBRAE, UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6633	9	<ul style="list-style-type: none"> 1 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES. 2 PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 2 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6634	10	<ul style="list-style-type: none"> 4 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 1 UNILATERAL RIB 14, STERNEBRAE NOT OSSIFIED. 3 STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. * 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED, METATARSALS NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 0 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6635	12	2 NO VISIBLE ABNORMALITIES. 1 - REDUCED OSSIFICATION OF THE PUBES. 3 REDUCED OSSIFICATION OF THE STERNEBRAE, RE- DUCED OSSIFICATION OF THE PUBES. 4 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 PUBES NOT OSSIFIED. 1 PUBES NOT OSSIFIED, STERNEBRAE NOT OSSIFIED.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 80 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6640	3	<ul style="list-style-type: none"> 1 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE.
6641	13	<ul style="list-style-type: none"> 5 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE PUBES. 1 UNILATERAL RIB 14, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE LUMBAR VERTEBRAL CENTRA, NON-FUSED OSSIFICATION CENTERS OF THE LUMBAR VERTEBRAL CENTRA., REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE. 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, NON-FUSED OSSIFICATION CENTERS OF THE LUMBAR VERTEBRAL CENTRA.
6642	4	<ul style="list-style-type: none"> 4 NO VISIBLE ABNORMALITIES.
6643	10	<ul style="list-style-type: none"> 8 NO VISIBLE ABNORMALITIES. -- 1 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 80 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6644	12	8 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, UNILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE PUBES.
6645	13	8 NO VISIBLE ABNORMALITIES. 2 UNILATERAL RIB 14. 1 BILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE HYOID BONE, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. * 1 REDUCED OSSIFICATION OF THE MAXILLA, REDUCED OSSIFICATION OF THE NASAL BONES, REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, BILATERAL RIB 14, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED, METATARSALS NOT OSSIFIED.
6646	10	6 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE. 1 REDUCED OSSIFICATION OF THE STERNEBRAE.
6647	12	2 NO VISIBLE ABNORMALITIES. 6 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE. 1 UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE STERNEBRAE.

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 80 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6648	7	5 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES.
6649	6	2 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. * 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE NASAL BONES, RE- DUCED OSSIFICATION OF THE HYOID BONE, STERNEBRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRUM, STERNEBRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 1 STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRUM, STERNEBRAE NOT OSSIFIED, REDUCED OSSI- FICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6650	11	8 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.
6651	10	2 NO VISIBLE ABNORMALITIES. 3 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE. 3 REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRUM.
6652	11	10 NO VISIBLE ABNORMALITIES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA.

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 80 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6653	10	<ul style="list-style-type: none"> 6 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA.
6654	9	<ul style="list-style-type: none"> 1 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, PUBES NOT OSSIFIED. 1 HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM. 1 STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM. * 1 SUPRAOCCIPITAL BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, LUMBAR VERTEBRAL CENTRA NOT OSSIFIED, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE LUMBAR VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 80 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6654 (CONTD)	9	* 1 HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, SUPRAOCCIPITAL BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE THORACIC AND LUMBAR VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6655	10	2 NO VISIBLE ABNORMALITIES. 5 REDUCED OSSIFICATION OF THE PUBES. 2 PUBES NOT OSSIFIED. 1 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE.
6656	11	5 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 5 REDUCED OSSIFICATION OF THE PUBES. 1 PUBES NOT OSSIFIED.
6657	11	1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 6 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 2 PUBES NOT OSSIFIED. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE PUBES, REDUCED OSSIFICATION OF THE STERNEBRAE.
6658	10	9 NO VISIBLE ABNORMALITIES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA.

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 80 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6659	9	<ul style="list-style-type: none">1 PUBES NOT OSSIFIED.4 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE.2 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, PUBES NOT OSSIFIED.1 REDUCED OSSIFICATION OF THE HYOID BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 250 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6665	10	10 NO VISIBLE ABNORMALITIES.
6666	11	11 NO VISIBLE ABNORMALITIES.
6667	8	2 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED.
6668	10	5 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES. 1 UNILATERAL RIB 14. 1 BILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES. 1 UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES.
6669	10	10 NO VISIBLE ABNORMALITIES.
6670	10	8 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES.
6671	9	3 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 250 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6672	8	8 NO VISIBLE ABNORMALITIES.
6673	11	9 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE PUBES. 1 BILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES.
6675	10	3 REDUCED OSSIFICATION OF THE PUBES. 3 PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.
6676	11	8 NO VISIBLE ABNORMALITIES. 1 BILATERAL RIB 14. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 REDUCED OSSIFICATION OF THE STERNEBRAE.
6677	9	1 NO VISIBLE ABNORMALITIES. 5 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE PUBES, REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 250 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6678	8	<ul style="list-style-type: none"> 2 PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 2 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE LUMBAR VERTEBRAL CENTRA, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED.
6679	6	6 NO VISIBLE ABNORMALITIES.
6680	12	<ul style="list-style-type: none"> 1 NO VISIBLE ABNORMALITIES. 5 PUBES NOT OSSIFIED. 3 REDUCED OSSIFICATION OF THE PUBES. 2 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6681	11	<ul style="list-style-type: none"> 3 STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM. 2 PUBES NOT OSSIFIED, STERNEBRAE NOT OSSIFIED. 1 BILATERAL RIB 14, PUBES NOT OSSIFIED, STERNE- BRAE NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, RE- DUCED OSSIFICATION OF THE PUBES. 1 HYOID BONE NOT OSSIFIED, STERNEBRAE NOT OSSI- FIED, PUBES NOT OSSIFIED.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 250 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6681 (CONTD)	11	<ul style="list-style-type: none"> 1 STERNEBRAE NOT OSSIFIED, UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 STERNEBRAE NOT OSSIFIED, BILATERAL RIB 14, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6682	10	<ul style="list-style-type: none"> 4 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 3 REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.
6683	12	<ul style="list-style-type: none"> 1 NO VISIBLE ABNORMALITIES. 6 REDUCED OSSIFICATION OF THE PUBES. 3 PUBES NOT OSSIFIED. 2 REDUCED OSSIFICATION OF THE STERNEBRAE, RE- DUCED OSSIFICATION OF THE PUBES.
6684	11	11 NO VISIBLE ABNORMALITIES.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 750 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6690	10	9 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14.
6691	13	8 NO VISIBLE ABNORMALITIES. 1 BILATERAL RIB 14. 3 REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA.
6692	8	8 NO VISIBLE ABNORMALITIES.
6693	12	7 NO VISIBLE ABNORMALITIES. 3 UNILATERAL RIB 14. 2 BILATERAL RIB 14.
6694	8	4 NO VISIBLE ABNORMALITIES. 1 BILATERAL RIB 14. 1 REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE.
6695	10	10 NO VISIBLE ABNORMALITIES.
6696	11	8 NO VISIBLE ABNORMALITIES. 1 UNILATERAL RIB 14. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 750 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6697	11	8 NO VISIBLE ABNORMALITIES. 2 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. * 1 STERNEBRAE MALALIGNED.
6698	11	8 NO VISIBLE ABNORMALITIES. 2 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA. 1 REDUCED OSSIFICATION OF THE PUBES.
6699	10	6 NO VISIBLE ABNORMALITIES. 3 REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.
6700	10	1 REDUCED OSSIFICATION OF THE HYOID BONE, STERNEBRAE NOT OSSIFIED, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 3 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, BILATERAL RIB 14, ISCHIUM NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, BILATERAL RIB 14, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE HYOID BONE, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 750 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6700 (CONTD)	10	1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED, ISCHIUM NOT OSSIFIED.
6701	8	4 NO VISIBLE ABNORMALITIES. 2 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, UNILATERAL RIB 14, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE STERNEBRAE, UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE PUBES.
6702	9	2 STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHIUM, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE PUBES.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 750 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6703	10	7 NO VISIBLE ABNORMALITIES. 3 REDUCED OSSIFICATION OF THE PUBES.
6704	14	1 NO VISIBLE ABNORMALITIES. 5 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE. 5 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 1 REDUCED OSSIFICATION OF THE PUBES. 1 STERNEBRAE NOT OSSIFIED.
6706	13	2 NO VISIBLE ABNORMALITIES 4 REDUCED OSSIFICATION OF THE PUBES. 1 PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE STERNEBRAE. 2 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE. 2 REDUCED OSSIFICATION OF THE HYOID BONE, RE- DUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED. 1 REDUCED OSSIFICATION OF THE HYOID BONE, NON- FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, (PORTIONS OF PELVIC GIRDLE AND HIND EXTREMITIES DAMAGED DURING PREPARATION AND HANDLING, UNABLE TO EVALUATE COMPLETELY).
6707	4	1 NO VISIBLE ABNORMALITIES. 1 REDUCED OSSIFICATION OF THE STERNEBRAE, RE- DUCED OSSIFICATION OF THE PUBES. 1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE PUBES. 1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO.10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 750 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6708	8	<p>1 STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED.</p> <p>1 REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED.</p> <p>2 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE LUMBAR VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, ISCHium NOT OSSIFIED, PUBES NOT OSSIFIED, METATARSALS NOT OSSIFIED.</p> <p>1 STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHium, PUBES NOT OSSIFIED.</p> <p>1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, PUBES NOT OSSIFIED.</p> <p>* 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE HYOID BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, REDUCED OSSIFICATION OF THE ISCHium, PUBES NOT OSSIFIED, METATARSALS NOT OSSIFIED.</p> <p>* 1 REDUCED OSSIFICATION OF THE INTERPARIETAL BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, REDUCED OSSIFICATION OF THE MAXILLA, HYOID BONE NOT OSSIFIED, REDUCED OSSIFICATION OF THE LUMBAR VERTEBRAL CENTRA, STERNEBRAE NOT OSSIFIED, ISCHium NOT OSSIFIED, PUBES NOT OSSIFIED.</p>
6709	12	<p>2 REDUCED OSSIFICATION OF THE PUBES.</p> <p>5 PUBES NOT OSSIFIED, REDUCED OSSIFICATION OF THE STERNEBRAE.</p> <p>1 UNILATERAL RIB 14, REDUCED OSSIFICATION OF THE STERNEBRAE, PUBES NOT OSSIFIED.</p> <p>1 REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.</p> <p>1 NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.</p>

*Some of these findings not commonly encountered.

TABLE II-D-6 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-05

TABLE 3 (CONTINUED)

OBSERVATIONS RESULTING FROM EXAMINATION OF CLEARED SPECIMENS

DOSE - 750 PPM

<u>FEMALE NO.</u>	<u>NO. OF FETUSES EXAMINED</u>	<u>OBSERVATIONS</u>
6709 (CONTD)	12	<p>1 REDUCED OSSIFICATION OF THE HYOID BONE, REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE ISCHIUM, PUBES NOT OSSIFIED.</p> <p>1 REDUCED OSSIFICATION OF THE SUPRAOCCIPITAL BONE, NON-FUSED OSSIFICATION CENTERS OF THE THORACIC VERTEBRAL CENTRA, REDUCED OSSIFICATION OF THE STERNEBRAE, REDUCED OSSIFICATION OF THE PUBES.</p>

PART II - SECTION E
THREE-GENERATION REPRODUCTION IN RATS

DCPD

LBI PROJECT NO. 10734-07

SUMMARY

Two groups of 10 male and 20 female albino rats each (F0 generation) were given dicyclopentadiene (DCPD) in the diet at 80 or 750 ppm, with a similar group maintained as controls. The rats in each group were mated twice to produce the F1a and F1b litters.

The same number of F1b pups per sex per group were likewise mated to produce F2a and F2b pups, and the F2b animals were maintained to produce F3a and F3b litters. Analyses of the diet mixes indicated 87 and 92% of the desired concentrations were achieved, on the average, for the low- and high-dose levels, respectively.

For each generation in each group there were determined fertility indices, live-to-total pup ratios, mean litter sizes, pup survival indices and mean body weights at Day 4 post partum and at weaning. Gross necropsy observations were made of representative pups of all F1a litters, of the F3b litters, and of the parent rats. Body weights and food consumption were determined for parent rats at various intervals, also.

It is concluded no deleterious effects on reproductive processes or general condition of the rats were produced by DCPD in this study. Likewise, no evidence of dose-related teratologic effect was seen.

1. OBJECTIVE

The purpose of this study was to evaluate the effects of the test material, dicyclopentadiene (DCPD), on the reproductive processes of the albino rat through three consecutive generations, with two matings per generation. Test material was given in the diet at two concentrations.

2. MATERIAL

Refer to Part II - Section A.

3. EXPERIMENTAL DESIGN

Weanling albino rats [CRL:COB (SD) BR] were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan, and acclimated to laboratory conditions for 11 days at the Falls Church facility of the Department of Toxicology. They were started on study on May 22, 1977, after being randomly assigned to groups as follows:

<u>Group Number</u>	<u>Number of Animals</u>		<u>Dietary Concentration (ppm)</u>
	<u>Male</u>	<u>Female</u>	
1	10	20	0 (Control)
2	10	20	80
3	10	20	750

These rats, the F0 generation, were identified by ear tags and cage cards, and housed individually (except when mating) in shoe box cages on AB-SORB-DRI bedding. Food and water were provided ad libitum.

Fresh diets were prepared weekly by adding the appropriate quantity of DCPD, dissolved in 300 ml of corn oil, to 10 kg of Purina Laboratory Chow meal, and mixing for at least 15 minutes in a twin shell blender. Control diet was mixed with corn oil in the same fashion.

Because of the possible loss from the diet through volatility of DCPD, samples of each week's dietary batch were analyzed by the LBI Chemistry Department. The analytical method employed gas-liquid chromatography and was supplied by the sponsor and developed at LBI.

Seven weeks after starting on compound the rats were mated for the first time. For this purpose each male was caged with two females of its dose group for two weeks. At the end of this time the rats were returned to their respective cages and the females were allowed to litter.

One week after weaning the first litters (F1a pups), the F0 parents were remated, each male with a different pair of females from that of the first mating. One week after weaning the second litters (F1b pups), parent F0 rats were killed and a gross necropsy was performed on each.

One male and two female F1b pups from each litter (where possible) were selected to be the parents for the next generation, and were caged, fed and watered just as the F0 rats were maintained. When the F1b rats were approximately 100 days of age, they were mated to produce the F2a litters, and subsequently the F2b pups. Selected F2b pups in turn were used to produce the F3a and F3b litters.

At 4 and at 8-9 weeks, parent rats were weighed and their food consumption was estimated. These measurements were made again shortly before each mating. Daily observations were made of parent rats for mortality and general condition.

3. EXPERIMENTAL DESIGN (Continued)

For each litter the following observations were made:

Gross abnormalities of pups

Numbers of live and dead pups, and their mean body weight by sex at birth

Number per sex at Day 4 of lactation

Number per sex and body weights at Day 21 of lactation (weaning)

At Day 4 each litter was reduced to eight total pups, four per sex if possible. At weaning, gross necropsies were performed on approximately one-third of the first litters from all three generations, and on one-third of the F3b litters.

4. RESULTS

A. Diet Concentrations Found

Results of the DCPD weekly feed analyses are presented in a separate report from the LBI Department of Chemistry. Overall, the results showed a 69.3 ppm (87%) average value for the 80-ppm diet level, and 693 ppm (92%) for the 750-ppm diet level. Considering the volatility of DCPD, these results are thought to indicate reasonable achievement of the intended dietary concentrations.

B. First Generation (F0 Parents, F1a and F1b Offspring)

Detailed litter data, mean parent body weights and food consumption figures, and pertinent necropsy observations have been presented in numbered Tables of the Appendix for this and succeeding generations. Summaries of reproductive data have been incorporated in the text in lettered tables.

One F0 female (No. 5196, 80 ppm) was found dead at Week 28, but all other F0 rats survived their portion of the study in generally good condition. Body weight means and daily food consumption means are presented in Appendix Tables 1 and 2, respectively. Data for compound-treated groups were entirely comparable to control figures at each interval.

Table 3 gives litter data for F1a pups, and these are summarized in Text Table A. There is nothing in these data to distinguish DCPD-treated groups from control rats. Likewise, observations of the pups (Table 4) indicate nothing of importance in the three groups. One pup in a 80-ppm litter had an opaque left eye, and one pup in a 750-ppm litter had a crooked tail. Such isolated findings are not meaningful. Table 5 gives necropsy findings of F1a pups. No compound-related findings were reported.

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

SUMMARY OF FIRST GENERATION - FIRST MATING (F1a)

INDICES	DOSE (PPM)					
	0		80		750	
	RATIO	PERCENT	RATIO	PERCENT	RATIO	PERCENT
Male fertility (males producing litter/mated)	10/10	100	10/10	100	9/10	90
Female fertility (females producing litter/mated)	19/20	95	18/20	90	16/20	80
Gestation (females live litter/pregnant)	19/19	100	18/18	100	16/16	100
Newborn viability (live pups/total pups)	209/211	99	210/212	99	198/200	99
Pup viability (pups Day 4/pups Day 0)	205/209	98	207/210	99	194/198	98
Lactation (pups Day 21/pups Day 4)	140/140	100	140/140	100	128/128	100
PUP WEIGHT IN GRAMS (MEAN \pm SD)						
Day 0 males	7 \pm 0.66		7 \pm 0.68		7 \pm 0.60	
Day 0 females	7 \pm 0.75		6 \pm 0.59		6 \pm 0.72	
Day 21 males	52 \pm 5.4		50 \pm 5.6		48 \pm 5.1	
Day 21 females	49 \pm 4.6		46 \pm 5.1		46 \pm 4.7	
Sex ratio offspring (M/F) Day 0	110/101		111/101		94/106	
Live pups per litter (Mean \pm SD)	11 \pm 3.7		12 \pm 2.8		12 \pm 2.3	

^aAfter litters reduced.

TABLE II-E-8

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE B

SUMMARY OF F0 GENERATION - SECOND MATING - F1b

	DOSE (PPM)		80		750	
	RATIO	PERCENT	RATIO	PERCENT	RATIO	PERCENT
INDICES						
Male fertility (males producing litter/mated)	10/10	100	10/10	100	10/10	100
Female fertility (females producing litter/mated)	18/20	90	18/20	90	19/20	95
Gestation (females live litter/pregnant)	18/18	100	18/18	100	19/19	100
Newborn viability (live pups/total pups)	199/201	99	196/202	97	229/231	99
Pup viability (pups Day 4/pups Day 0)	193/199	97	187/196	95	216/229	94
Lactation (pups Day 21/pups Day 4) ^a	128/132	97	128/132	97	139/145	96

PUP WEIGHT IN GRAMS (MEAN \pm SD)

Day 0 males	6 \pm 1.1	5 \pm 1.6	6 \pm 0.61
Day 0 females	6 \pm 1.1	5 \pm 1.6	6 \pm 0.61
Day 21 males	46 \pm 7.8	47 \pm 8.8	42 \pm 6.8
Day 21 females	44 \pm 6.9	44 \pm 8.2	40 \pm 5.7
Sex ratio offspring (M/F) Day 0	100/101	94/108	108/123
Live pups per litter (Mean \pm SD)	11 \pm 3.5	11 \pm 3.7	12 \pm 3.2

^aAfter litters reduced.

4. RESULTS (Continued)

Tables 6 and 7 present, respectively, litter data and pup observations for the Flb offspring, with the litter data summarized in Table B. All groups were comparable to one another with respect to both litter data and pup observations. Again, the single instance of a pup in the 80-ppm group with an abnormality (a deformed hind foot) cannot be considered meaningful.

Necropsy findings of F0 parents (Table 8) indicate no dose-related changes.

C. Second Generation (Flb Parents, F2a and F2b Offspring)

Tables 9 and 10 show mean body weights and daily food consumption figures, respectively, for Flb parent rats. At all intervals, rats in the compound-treated groups weighed as much as or more than the controls, except for the 80-ppm females at 20 weeks (just prior to the second mating). In this instance, the slightly lower mean body weight was not statistically significant. Similarly, food consumption means were comparable among the groups, except that in both males and females of the 750-ppm group, the reductions at 20 weeks in food intake were statistically significant ($p < 0.05$, Student's t-test).

F2a litter data, pup general observations and pup necropsy observations are presented in Tables 11, 12 and 13, respectively; litter data are summarized in Table C. Except for reduced female fertility in the 750-ppm group (discussed below) litter data in all groups were comparable. Similarly, no general or necropsy findings of significance were recorded, but one male pup in the 80-ppm group was found to have had hydrocephalus.

F2b litter data and pup observations are presented in Tables 14 and 15, respectively, with litter data summarized in Table D. General pup observations were unexceptional, but, as with the F2a litters, fertility in the 750-ppm females was reduced. However, neither the 70% figure for the F2a's nor the 85% for the F2b's was statistically significantly different (Chi-square test) from the control index (95% in each instance). In addition, it may be noted that male No. 7349 in the 750-ppm group failed to sire a litter in either mating, and could thus be responsible for the lack of litters from two of six and two of three non-productive females at the first and second matings, respectively. It is therefore concluded that the apparently lowered fertility of the high-dose females at each mating is not related to compound administration.

At necropsy, no gross lesions were found in the Flb parent rats.

TABLE II-E-9

LITTON BIONETICS, INC.
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TABLE C

SUMMARY OF F1b GENERATION - FIRST MATING - F2a

	DOSE (PPM)		80		750	
	RATIO	PERCENT	RATIO	PERCENT	RATIO	PERCENT
INDICES						
Male fertility (males producing litter/mated)	10/10	100	10/10	100	9/10	90
Female fertility (females producing litter/mated)	19/20	95	18/20	90	14/20	70
Gestation (females live litter/pregnant)	19/19	100	18/18	100	14/14	100
Newborn viability (live pups/total pups)	241/242	100	209/216	97	162/162	100
Pup viability (pups Day 4/pups Day 0)	237/241	98	196/209	94	159/162	98
Lactation (pups Day 21/pups Day 4) ^a	147/150	98	135/139	97	107/109	98
PUP WEIGHT IN GRAMS (MEAN \pm SD)						
Day 0 males	6 \pm 0.90		6 \pm 0.84		6 \pm 0.83	
Day 0 females	6 \pm 0.79		6 \pm 0.92		6 \pm 0.95	
Day 21 males	44 \pm 5.9		46 \pm 6.4		44 \pm 5.5	
Day 21 females	41 \pm 5.3		43 \pm 6.6		42 \pm 5.3	
Sex ratio offspring (M/F) Day 0	111/131 ^b		94/122 ^b		84/78 ^b	
Live pups per litter (Mean \pm SD)	13 \pm 2.6		12 \pm 2.7		12 \pm 2.7	

^aAfter litters reduced.
^bSome pups mis-sexed.

4. RESULTS (Continued)

D. Third Generation (F2b Parents, F3a and F3b Offspring)

Tables 16 and 17 show, respectively, body weights and food consumption data for the F2b parents. No meaningful differences between groups at the various intervals were seen with respect to these observations.

Litter data (Table 18), pup observations (Table 19) and pup necropsy observations (Table 20) for the F3a offspring are appended. The litter data are summarized in Table E. There was nothing in these records to suggest a compound-related effect, and although female fertility was only 80% and 83% in the DCPD-treated groups, control fertility was worse, only 65%.

Tables 21, 22 and 23 show, respectively, the litter observations, pup general observations and pup necropsy findings for the F3b offspring. Table F summarizes the litter data, and it may be seen that all groups were generally comparable with respect to the various indices. Female fertility percentages were 85, 80 and 83 for the controls, 80- and 750-ppm groups, respectively. The differences are not meaningful. A slight reduction in mean pup weight at weaning (compared to controls) was noted in each compound-treated group, that for the high-dose female pups being statistically significant ($p < 0.05$; Student's t-test). The low-dose female pup weight, while numerically the same as that of the 750-ppm weanlings, was not statistically significant. Since mean weanling pup weights in the other generations (Tables A, B, C, D and E) were not appreciably different among the groups involved, it is felt that the F3b differences are fortuitous.

Pup general observations and necropsy observations showed nothing to indicate compound-related effects. Similarly, necropsy findings of the F2b parents (Table 24) were unexceptional.

Thirty days after transmittal of this report, original data from the Department of Toxicology will be transferred to the LBI Archivist, 5516 Nicholson Lane, Kensington, Maryland, for distribution to the proper repositories. A copy of this report was reviewed by the LBI Quality Assurance Unit.

TABLE 11-E-10

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE D

SUMMARY OF F1b GENERATION - SECOND MATING - F2b

	DOSE (PPM)			
	0		80	
	RATIO	PERCENT	RATIO	PERCENT
Male fertility (males producing litter/mated)	10/10	100	10/10	100
Female fertility (females producing litter/mated)	19/20	95	19/20	95
Gestation (females live litter/pregnant)	19/19	100	19/19	100
Newborn viability (live pups/total pups)	263/266	99	286/287	100
Pup viability (pups Day 4/pups Day 0)	250/263	95	280/286	98
Lactation (pups Day 21/pups Day 4) ^a	149/151	99	149/152	98

PUP WEIGHT IN GRAMS (MEAN \pm SD)

Day 0 males	6 \pm 0.84	6 \pm 0.63	6 \pm 0.54
Day 0 females	6 \pm 0.75	6 \pm 0.52	6 \pm 0.66
Day 21 males	45 \pm 6.8	48 \pm 7.2	51 \pm 6.6
Day 21 females	43 \pm 7.4	46 \pm 6.6	48 \pm 6.6
Sex ratio offspring (M/F) Day 0	121/145	146/141	119/116
Live pups per litter (Mean \pm SD)	14 \pm 2.5	15 \pm 1.6	14 \pm 1.4

^aAfter litters reduced.

TABLE II-E-11

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE E

SUMMARY OF F2b GENERATION - FIRST MATING - F3a

INDICES	DOSE (PPM)			
	0		80	
	RATIO	PERCENT	RATIO	PERCENT
Male fertility (males producing litter/mated)	9/10	90	10/10	100
Female fertility (females producing litter/mated)	13/20	65	16/20	80
Gestation (females live litter/pregnant)	13/13	100	16/16	100
Newborn viability (live pups/total pups)	162/163	99	195/196	99
Pup viability (pups Day 4/pups Day 0)	156/162	96	187/195	96
Lactation (pups Day 21/pups Day 4) ^a	92/100	92	118/118	100
<u>PUP WEIGHT IN GRAMS (MEAN ± SD)</u>				
Day 0 males	6 ± 0.77		6 ± 1.3	
Day 0 females	7 ± 0.80		5 ± 1.2	
Day 21 males	46 ± 5.8		46 ± 4.7	
Day 21 females	45 ± 7.6		42 ± 4.2	
Sex ratio offspring (M/F) Day 0	81/82		103/93	
Live pups per litter (Mean ± SD)	12 ± 3.3		12 ± 3.9	
			108/98	
			14 ± 2.0	

^aAfter litters reduced.

TABLE II-E-12

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE F

SUMMARY OF F2b GENERATION - SECOND MATING - F3b

	DOSE (PPM)			
	0		80	
	<u>RATIO</u>	<u>PERCENT</u>	<u>RATIO</u>	<u>PERCENT</u>
<u>INDICES</u>				
Male fertility (males producing litter/mated)	9/10	90	10/10	100
Female fertility (females producing litter/mated)	17/20	85	16/20	80
Gestation (females live litter/pregnant)	17/17	100	16/16	100
Newborn viability (live pups/total pups)	211/215	98	206/213	97
Pup viability (pups Day 4/pups Day 0)	207/211	98	206/206	100
Lactation (pups Day 21/pups Day 4) ^a	134/135	99	127/128	99
<u>PUP WEIGHT IN GRAMS (MEAN ± SD)</u>				
Day 0 males	6 ± 0.79		7 ± 0.98	7 ± 0.83
Day 0 females	6 ± 0.64		6 ± 0.87	6 ± 0.83
Day 21 males	49 ± 10		44 ± 11	43 ± 11
Day 21 females	48 ± 9.3		41 ± 12	41 ± 9.5*
Sex ratio offspring (M/F) Day 0	93/122		107/106	93/98
Live pups per litter (Mean ± SD)	12 ± 2.7		13 ± 2.5	13 ± 2.8

^aAfter litters reduced.

*p<0.05 compared to control: Student's t-test.

5. CONCLUSION

Dietary administration of dicyclopentadiene (DCPD) at nominal concentrations of 80 and 750 ppm to three successive generations of male and female albino rats had no deleterious effects on reproductive performance or general condition of the animals, in comparison to performance of control rats maintained concurrently. Analyses of the diet throughout the study indicated actual DCPD concentrations averaged 87 and 92% of those intended for the 80- and 750-ppm levels, respectively. No evidence of dose-related teratogenic effect was seen in pups of any generation.

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TABLE II-E-13

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 1

MEAN BODY WEIGHTS (G) OF FO RATS

DOSE (PPM)	SEX		WEEK			
			4	8	11	19
0	M	MEAN	313	413	447	517
		SD	22	40	39	40
		SE	7.1	13	12	13
		N	10	10	10	10
80	M	MEAN	319	426	465	538
		SD	15	24	28	38
		SE	4.6	7.5	9.0	12
		N	10	10	10	10
750	M	MEAN	312	416	456	529
		SD	16	32	34	52
		SE	5.2	10	11	17
		N	10	10	10	10
0	F	MEAN	199	237	252	290
		SD	10	17	20	23
		SE	2.2	3.9	4.9	5.1
		N	20	20	20	20
80	F	MEAN	206	249	275	295
		SD	18	27	46	32
		SE	4.1	6.0	10	7.1
		N	20	20	20	20
750	F	MEAN	206	242	264	293
		SD	20	36	27	31
		SE	4.4	8.0	6.1	7.0
		N	20	20	20	20

TABLE II-E-14

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 2

MEAN FOOD CONSUMPTION (G) IN FO RATS

DOSE (PPM)	SEX		WEEK			
			4	8	11	19
0	M	MEAN	22	22	21	19
		SD	8.4	3.0	3.0	2.3
		SE	3.0	1.0	0.99	0.77
		N	8	10	9	9
80	M	MEAN	24	23	22	21
		SD	3.0	2.2	2.4	4.0
		SE	0.96	0.74	0.75	1.3
		N	10	9	10	10
750	M	MEAN	20	23	22	20
		SD	4.2	2.4	2.7	3.0
		SE	1.3	0.81	0.86	0.99
		N	10	9	10	9
0	F	MEAN	18	19	16	18
		SD	3.8	5.4	4.1	2.6
		SE	0.84	1.2	0.91	0.64
		N	20	19	20	17
80	F	MEAN	18	18	16	19
		SD	3.3	2.4	3.3	3.7
		SE	0.74	0.55	0.74	0.85
		N	20	19	20	19
750	F	MEAN	16	19	16	19
		SD	3.8	4.7	4.9	4.2
		SE	0.87	1.1	1.1	0.95
		N	19	19	20	20

TABLE II-E-15

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 3

RESULTS OF FIRST GENERATION - FIRST MATING (F1a)

DOSE - 0 PPM (CONTROL)

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)	
		LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	LIVE PUPS	DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	LITTER REDUCED MALE FEMALE	DEATHS DAY 5-21	LIVE PUPS	SEX MALE FEMALE
5150	5140	13	0	4 9	7 7	0 0	13	4 9	4 4	0 0	8	4 4
5151	5141	14	0	8 6	7 7	0 0	14	8 6	4 4	0 0	8	4 4
5152	5142	11	1	9 3	8 8	0 0	11	9 2	6 2	0 0	8	4 4
5153	5143	9	0	5 4	8 8	0 0	9	5 4	4 4	0 0	8	4 4
5154	5144	14	0	6 8	6 6	0 0	14	6 8	4 4	0 0	8	4 4
5155	5145	11	0	6 5	7 7	0 0	11	6 5	4 4	0 0	8	4 4
5156	5146	15	0	8 7	8 8	0 0	15	8 7	4 4	0 0	8	4 4
5157	5147	13	0	3 1	7 7	0 0	13	3 1	3 1	0 0	4	3 1
5158	5148	14	0	5 8	6 6	0 0	14	5 8	4 4	0 0	8	4 4
5159	5149	14	0	8 6	7 7	0 0	14	8 6	4 4	0 0	8	4 4
5160	5150	9	0	3 6	7 7	0 0	9	3 6	3 5	0 0	8	4 4
5161	5151	1	1	2 0	7 7	0 0	1	1 0	1 0	0 0	1	1 0
5162	5152	9	0	6 3	8 8	1 1	8	5 3	5 3	0 0	8	5 3
5163	5153	8	0	4 4	8 8	1 1	7	4 3	4 3	0 0	7	4 3
5164	5154	13	0	7 6	7 6	2 2	11	6 5	4 4	0 0	8	4 4
5165	5155	13	0	6 7	7 7	0 0	13	6 7	4 4	0 0	8	4 4
5166	5156	11	0	6 5	7 7	0 0	11	6 5	4 4	0 0	8	4 4
5167	5157	13	0	7 6	7 7	0 0	13	7 6	4 4	0 0	8	4 4
5168	5158	13	0	7 6	7 7	0 0	13	7 6	4 4	0 0	8	4 4
5169	5159	13	0	7 6	7 7	0 0	13	7 6	4 4	0 0	8	4 4
TOTAL		209	2	110 101		4	205	107 98	74 66	0	140	74 66
MEAN		11			7		11				7	
SD		3.7			0.66		3.7					
SE		0.64			0.40		0.65					
N		19			19		19					

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 3 (CONTINUED)

RESULTS OF FIRST GENERATION - FIRST MATING (Fla)

DOSE ~ 80 PPM

[illegible]

TABLE II-E-15 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 3 (CONTINUED)

RESULTS OF FIRST GENERATION - FIRST MATING (Fl_a)

DOSE - 750 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0		DAY 4		DAY 21		DAY 21		DAY 21		DAY 21	
		LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (G)	DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	LITTER REDUCED MALE FEMALE	DEATHS DAY 5-21	LIVE PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (G)
					MALE FEMALE								MALE FEMALE
5210	5200	-	-	-	-	-	-	-	-	-	-	-	-
5211	5201	11	0	5	6	0	11	5	4	0	8	4	44
5212	5202	10	0	5	6	0	10	5	4	0	8	4	45
5213	5203	9	1	5	7	0	9	5	4	0	8	4	44
5214	5204	9	0	3	7	0	9	3	5	0	8	3	50
5215	5205	16	0	8	7	0	16	8	4	0	8	4	49
5216	5206	10	0	5	6	2	8	4	4	0	8	4	50
5217	5207	12	0	7	7	0	12	7	4	0	8	4	49
5218	5208	13	0	5	7	0	13	5	4	0	8	4	45
5219	5209	14	0	7	7	1	13	6	4	0	8	4	49
5220	5210	-	-	-	-	-	-	-	-	-	-	-	37
5221	5211	11	0	3	7	0	11	3	5	0	8	3	54
5222	5212	14	0	8	7	0	14	8	4	0	8	4	50
5223	5213	11	1	7	7	0	11	6	4	0	8	4	57
5224	5214	15	0	5	6	0	15	5	4	0	8	4	45
5225	5215	14	0	7	7	0	14	7	4	0	8	4	56
5226	5216	-	-	-	-	-	-	-	-	-	-	-	51
5227	5217	-	-	-	-	-	-	-	-	-	-	-	50
5228	5218	15	0	5	7	1	14	5	4	0	8	4	49
5229	5219	14	0	9	7	0	14	9	4	0	8	4	45
TOTAL		198	2	94	106	4	194	91	62	0	128	62	48
MEAN		12			7		12				8		46
SD		2.3			0.60		2.4						5.1
SE		0.57			0.15		0.59						1.1
N		16			16		16						16

TABLE II-E-16

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 4

PUP OBSERVATIONS (F1a)

DOSE (PPM)	FEMALE NUMBER	DAY OF LACTATION	OBSERVATIONS
0	5150	0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
		0	ONE MALE - SMALL SCAB ON SURFACE OF RIGHT EYELID.
	5151	0	ONE MALE - HEMATOMA TOP OF HEAD.
	5154	0	FIVE FEMALES - HEMATOMAS MID-DORSAL THORACIC REGION.
	5156	0	ONE MALE - LESION BETWEEN RIGHT EYE AND RIGHT EAR.
	5159	0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
	5160	0	THREE MALES - HEMATOMAS MID-DORSAL THORACIC REGION.
		0	ONE FEMALE - SMALL LESION MID-DORSAL LUMBAR REGION.
	5162	0	ONE MALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5165	4	ONE MALE - FOUND DEAD; SMALL; YELLOW COLOR SKIN.
		4	ONE FEMALE - APPEARS SMALL.
	5167	0	ONE FEMALE - HEMATOMA HEAD.
		0	ONE FEMALE - HEMATOMA LEFT HIND FOOT.
	5168	0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
80	5181	0	ONE MALE - FOUND DEAD.
		0	ONE FEMALE - LACERATION BETWEEN EYES.
		0	ONE FEMALE - LACERATION ABOVE NOSE.
	5184	0	ONE MALE - HEMATOMAS LEFT SIDE MOUTH; LEFT SIDE NOSE.
	5185	0	ONE FEMALE - HEMATOMA RIGHT SIDE NOSE.
	5186	0	ONE MALE - HEMATOMA NOSE.
	5187	0	ONE FEMALE - HEMATOMA NOSE.
	5188	0	ONE FEMALE - ABDOMINAL SKIN YELLOW.
	5191	0	ONE MALE - HEMATOMA LEFT HIND FOOT.
		0	ONE MALE - HEMATOMA MID-DORSAL THORACIC REGION.
	5196	0	ONE MALE - HEMATOMA MID-DORSAL THORACIC REGION.
750	5199	21	ONE FEMALE - LEFT EYE OPAQUE; SMALLER THAN RIGHT EYE.
	5211	0	ONE FEMALE - SMALL LESION MID-DORSAL THORACIC REGION.
	5215	0	ONE MALE - SCAB OVER NOSE.
		0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
	5216	0	ONE MALE - HEMATOMA EXTENSIVE OVER HIND LIMBS; SACRUM.
		0	ONE MALE - HEMATOMA HEAD.
	5218	0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
	5219	0	ONE MALE - HEMATOMA UNDER JAW.
		4	ONE MALE - CROOKED TAIL.
	5225	0	ONE MALE - HEMATOMA MID-DORSAL THORACIC REGION.
		0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
	5228	0	TWO MALES - HEMATOMAS FACE.

TABLE II-E-17

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 5

PUP NECROPSY OBSERVATIONS (F1a)

DOSE (PPM)	FEMALE NUMBER	NUMBER OF PUPS	OBSERVATIONS
0	5150	8	NO VISIBLE ABNORMALITIES.
	5152	8	ONE MALE - PORTIONS OF LUNGS DARK RED.
	5153	8	NO VISIBLE ABNORMALITIES.
	5156	8	NO VISIBLE ABNORMALITIES.
	5160	8	NO VISIBLE ABNORMALITIES.
	5168	8	NO VISIBLE ABNORMALITIES.
80	5181	8	ONE MALE, ONE FEMALE - PORTIONS OF LUNGS DARK RED.
	5182	6	NO VISIBLE ABNORMALITIES.
	5184	8	NO VISIBLE ABNORMALITIES.
	5187	8	NO VISIBLE ABNORMALITIES.
	5190	7	NO VISIBLE ABNORMALITIES.
	5198	8	NO VISIBLE ABNORMALITIES.
750	5211	8	NO VISIBLE ABNORMALITIES.
	5212	8	TWO MALES, TWO FEMALES - VERY DARK LUNGS.
	5214	8	NO VISIBLE ABNORMALITIES.
	5218	8	NO VISIBLE ABNORMALITIES.
	5221	8	NO VISIBLE ABNORMALITIES.
	5229	8	NO VISIBLE ABNORMALITIES.

TABLE II-E-18

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 6

RESULTS OF F0 GENERATION - SECOND MATING. (F1b)

DOSE - 0 PPM (CONTROL.)

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)	
		LIVE PUPS	DEAD PUPS	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 1-4	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 5-21	SEX MALE-FEMALE	LIVE PUPS	LITTER REDUCED MALE-FEMALE
5150	5149	10	0	6 4	7 7	0 0	6 4	10 10	1 1	3 4	7 7	4 4
5151	5148	14	0	7 7	7 7	0 0	7 7	14 14	1 1	3 4	7 7	4 4
5152	5147	16	0	10 6	6 6	2 2	0 6	14 14	1 1	4 4	7 7	4 4
5153	5146	11	0	6 5	7 6	0 0	6 5	11 9	0 0	4 4	8 8	4 4
5154	5145	10	0	4 6	4 4	1 1	4 5	9 9	1 1	4 4	7 7	4 4
5155	5144	14	0	5 9	6 6	0 0	5 9	14 14	0 0	4 4	8 8	4 4
5156	5143	13	0	7 6	6 5	0 0	7 6	13 13	0 0	4 4	8 8	4 4
5157	5142	6	0	4 2	7 7	0 0	4 2	6 6	0 0	4 4	8 8	4 4
5158	5141	14	0	5 9	5 4	1 1	4 5	13 13	0 0	4 4	8 8	4 4
5159	5140	11	0	7 4	6 6	0 0	7 4	11 11	0 0	4 4	8 8	4 4
5160	5139	12	2	6 6	6 6	1 1	5 6	11 5	0 0	4 4	8 8	4 4
5161	5138	5	0	2 3	7 7	0 0	2 3	5 9	0 0	2 3	5 5	2 3
5162	5137	9	0	7 2	6 6	0 0	7 2	9 11	0 0	4 4	6 6	4 4
5163	5136	11	0	6 5	8 8	0 0	6 5	11 13	0 0	4 4	8 8	4 4
5164	5135	14	0	4 10	4 4	1 1	4 9	13 12	0 0	4 4	8 8	4 4
5165	5134	12	0	5 7	8 7	0 0	5 7	12 14	0 0	4 4	8 8	4 4
5166	5133	14	0	8 6	7 6	0 0	8 6	14 14	0 0	4 4	8 8	4 4
5167	5132	3	0	1 2	7 6	0 0	1 2	3 3	0 0	1 2	3 3	1 2
5168	5131	199	2	100 101	6 6	6 6	96 97	193 11	4 4	65 63	128 7	65 63
5169	5130	11	0	7 4	7 7	0 0	7 4	11 11	0 0	4 4	8 8	4 4
TOTAL		199	2	100 101	6 6	6 6	96 97	193 11	4 4	65 63	128 7	65 63
MEAN		3.5			1.1	1.1		3.3				
SD		0.8			0.26	0.26		0.78				
N		18			18	18		18				

TABLE II-E-18 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 6 (CONTINUED)

RESULTS OF F0 GENERATION - SECOND MATING (F1b)

DOSE - 80 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)			
		LIVE PUPS	DEAD PUPS	SEX MALE-FEMALE	MEAN PUP WEIGHT (G) MALE-FEMALE	DEATHS DAY 1-4	LIVE PUPS	SEX MALE-FEMALE	LITTER REDUCED MALE-FEMALE	DEATHS DAY 5-21	LIVE PUPS	SEX MALE-FEMALE	MALE	FEMALE
5180	5179	15	0	8 7	8 7	1 0	14 12	7 6	4 4	0 0	8 8	4 4	59	56
5181	5178	12	0	6 6	7 6	0 0	12 7	6 6	4 4	0 0	8 8	4 4	39	36
5183	5177	7	0	2 5	8 8	0 0	7 11	5 5	2 4	0 0	6 8	2 4	56	52
5184	5177	11	0	6 5	4 3	0 0	11 15	6 5	4 4	0 0	8 8	4 4	43	40
5185	5176	15	0	4 11	6 6	0 0	15 11	4 7	4 4	0 0	8 8	4 4	32	31
5186	5176	11	1	5 7	4 3	0 0	11 12	4 7	4 4	0 0	8 8	4 4	46	47
5187	5175	12	0	6 6	5 5	0 0	12 11	6 6	4 4	0 0	8 8	4 4	48	43
5188	5175	12	0	5 7	6 6	1 1	11 11	5 6	4 4	0 0	8 8	4 4	51	50
5189	5174	-	-	-	-	-	-	-	-	-	-	-	-	-
5190	5174	10	0	6 4	8 7	0 0	10 11	6 4	4 4	0 0	8 8	3 5 ^a	53	45
5191	5173	11	1	9 3	4 4	0 0	11 9	2 2	6 2	0 0	8 8	6 2	48	44
5173	5173	11	0	6 5	6 6	2 2	9 3	5 4	4 4	2 2	6 6	3 3	56	53
5193	5172	3	0	3 0	4 4	0 0	3 3	0 0	3 0	0 0	3 3	3 0	55	52
5194	5172	14	0	6 8	6 6	0 0	14 12	8 6	4 4	0 0	8 8	4 4	55	52
5195	5171	14	1	6 9	3 3	2 2	12 14	6 6	4 4	0 0	8 8	4 4	32	33
5196	5171	16	1	5 12	4 3	2 2	14 3	5 9	4 4	0 0	8 8	4 4	41	37
5197	5170	3	0	1 2	5 5	0 0	3 11	2 2	1 2	0 0	3 3	1 2	56	56
5198	5170	11	0	4 7	4 4	0 0	11 7	4 7	4 4	2 2	6 6	3 3	37	38
5199	5170	8	2	6 4	4 4	1 1	7 7	5 2	4 4	0 0	8 8	4 4	38	35
TOTAL		196	6	94 108		9	187	90 97	68 64	4	128	65 63	47	44
MEAN		11		5 5		10	3.5				7		8.8	8.2
SD		3.7		1.6 1.6			0.82						2.1	2.0
SE		0.87		0.4 0.4									18	17
N		18		18 17		18								

^aPup mfs-sexad.

TABLE II-E-18 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-07

TABLE 6 (CONTINUED)

RESULTS OF F0 GENERATION - SECOND MATING (F1b)

DOSE - 750 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)			
		LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	LIVE PUPS	DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	LITTER REDUCED MALE FEMALE	DEATHS DAY 5-21	LIVE PUPS	SEX MALE FEMALE	MALE	FEMALE
5210	5209	13	0	4 9	7 6	0	13	4 9	4 4	0	8	4 4	39	40
5211		18	0	7 11	6 6	1	17	6 11	4 4	0	8	4 4	50	44
5212	5208	13	0	6 7	6 6	0	13	7 7	4 4	0	8	4 4	44	44
5213		6	0	2 4	6 6	0	6	4 4	2 2	0	6	2 2	50	46
5214	5207	8	0	2 6	7 7	0	8	6 6	6 6	0	8	2 2	40	39
5215		16	0	6 10	7 6	0	16	10 6	4 4	0	8	4 4	27	24
5216	5206	11	1	7 5	6 6	0	11	5 6	4 4	0	8	4 4	46	44
5217		11	1	8 4	5 5	0	11	4 7	4 4	2	6	4 4	39	37
5218	5205	13	0	7 6	5 5	0	13	6 7	4 4	0	8	4 4	33	32
5219		11	0	6 5	7 7	1	10	5 6	4 4	2	6	3 3	38	40
5220	5204	-	-	-	-	-	-	-	-	-	-	-	-	-
5221		14	0	7 7	7 6	0	14	7 7	4 4	0	8	4 4	49	47
5222	5203	17	0	8 9	6 6	1	16	8 8	4 4	0	8	4 4	49	44
5223		11	0	7 4	6 6	0	11	7 4	4 4	0	8	4 4	38	39
5224	5202	15	0	6 9	7 7	0	15	9 6	4 4	0	8	4 4	51	44
5225		12	0	3 9	7 7	8	4	3 1	3 1	0	8	3 1	35	37
5226	5201	10	0	5 5	6 6	1	9	5 4	4 4	0	8	4 4	38	36
5227		13	0	7 6	6 6	0	13	6 7	4 4	1	7	4 3	34	33
5228	5200	10	0	6 4	7 6	1	9	5 4	4 4	1	7	4 3	45	41
5229		7	0	4 3	7 7	0	7	4 3	4 3	0	7	4 3	46	44
TOTAL		229	2	108 123	6 6	13	216	102 114	71 74	6	139	68 71	42	40
MEAN		12			0.61		11				7		6.8	5.7
SD		3.2			0.14		3.6						1.6	1.3
SE		0.73			0.19		0.02						19	19
N		19			19		19							

TABLE II-E-19

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 7

PUP OBSERVATIONS (F1b)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATIONS</u>
0	5152	6	ONE FEMALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5154	4	ONE FEMALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5159	0	TWO MALES - HEMATOMA MID-DORSAL THORACIC REGION.
	5160	0	ONE MALE - HEMATOMA RIGHT SIDE OF FACE; ONE MALE - HEMATOMA MID-DORSAL THORACIC REGION.
	5166	0	ONE FEMALE - HEMATOMA LEFT DORSAL THORACIC REGION.
80	5180	0	ONE FEMALE - HEMATOMA MID-DORSAL THORACIC REGION.
		4	ONE MALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5185	0	ONE FEMALE - HEMATOMA RIGHT HIND LEG.
	5186	0	ONE MALE - FOUND DEAD.
	5188	0	ONE FEMALE - HEMATOMA ON FACE.
	5191	0	ONE FEMALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5195	0	ONE FEMALE - FOUND DEAD; MISSING TAIL; RIGHT HIND FOOT DEFORMED.
	5196	0	ONE FEMALE - FOUND DEAD; HEMATOMA RIGHT SIDE OF FACE.
750	5199	0	ONE MALE - FOUND DEAD; ONE FEMALE - FOUND DEAD; ONE MALE - HEMATOMA ENTIRE ABDOMEN AND INGUINAL REGION.
	5217	0	ONE PUP FOUND DEAD; PARTIALLY CANNIBALIZED.
		11	ONE MALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5222	4	ONE MALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	5225	4	ALL PUPS VERY SMALL.
	5227	0	ONE FEMALE - HEMATOMA LEFT HIND FOOT.
	5228	0	ONE MALE - LABORED RESPIRATION.

TABLE II-E-20

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 8

ADULT NECROPSY OBSERVATIONS (FO)

<u>DOSE</u> <u>(PPM)</u>	<u>SEX</u>	<u>ANIMAL</u> <u>NUMBER</u>	<u>OBSERVATIONS</u>
0	M	5161	NO VISIBLE ABNORMALITIES.
	F		FIRM MASS (1 CM ²) LEFT VENTRAL THORACIC REGION.
80	M	5181	NO VISIBLE ABNORMALITIES.
	F	5199	CLEAR FLUID-FILLED SAC (0.5 CM ²) ON LEFT OVARY. STONE (1 X 0.5 CM) IN URETER; STONE (1 X 0.5 CM) IN BLADDER.
750	M	5207	HAIR LOSS; DISCHARGE LEFT EYE.
	F		NO VISIBLE ABNORMALITIES.

TABLE II-E-21

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 9

MEAN BODY WEIGHTS (G) OF F1b RATS

DOSE (PPM)	SEX		WEEK			
			4	9	11	20
0	M	MEAN	281	416	432	513
		SD	29	31	39	45
		SE	9.0	9.8	12	14
		N	10	10	10	10
80	M	MEAN	290	447	472	574
		SD	36	34	35	51
		SE	12	11	11	16
		N	10	10	10	10
750	M	MEAN	281	429	449	569
		SD	27	36	29	48
		SE	8.4	12	9.2	15
		N	10	10	10	10
0	F	MEAN	183	239	246	291
		SD	16	14	16	18
		SE	3.6	3.1	3.6	4.0
		N	20	20	20	20
80	F	MEAN	183	246	251	283
		SD	22	22	23	24
		SE	5.0	4.9	5.2	5.4
		N	20	20	20	20
750	F	MEAN	185	250	256	309
		SD	20	24	24	26
		SE	4.4	5.3	5.3	5.7
		N	20	20	20	20

TABLE II-E-22

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 10

MEAN FOOD CONSUMPTION (G) IN F1b RATS

DOSE (PPM)	SEX		WEEK			
			4	9	11	20
0	M	MEAN	25	26	26	32
		SD	3.8	3.0	3.7	5.7
		SE	1.2	1.1	1.4	1.8
		N	10	8	7	10
80	M	MEAN	26	28	27	30
		SD	3.2	1.3	1.7	3.2
		SE	1.3	0.46	0.55	1.0
		N	6	8	10	10
750	M	MEAN	25	25	25	25*
		SD	1.9	1.9	0.58	3.2
		SE	0.67	0.63	0.20	1.0
		N	8	9	8	10
0	F	MEAN	22	22	23	29
		SD	4.6	4.2	6.0	4.0
		SE	1.1	0.89	1.4	1.0
		N	17	18	18	16
80	F	MEAN	25	25	26	33
		SD	2.6	3.6	4.4	6.1
		SE	0.91	0.96	1.2	1.4
		N	8	14	13	18
750	F	MEAN	21	22	21	24*
		SD	4.9	4.3	5.0	5.9
		SE	1.3	1.0	1.2	1.6
		N	15	17	19	14

*p<0.05 as compared to controls: Student's t-test.

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

RESULTS OF F1b GENERATION - FIRST MATING (F2a)

DOSE - 0 PPM (CONTROL)

[illegible]

89999 mts-south.

TABLE II-E-23 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 11 (CONTINUED)

RESULTS OF F1b GENERATION - FIRST MATING (F2a)

DOSE - 80 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)		
		LIVE PUPS	DEAD PUPS	SEX MALE-FEMALE	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 1-4	LITTER REDUCED MALE-FEMALE	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 5-21	SEX MALE-FEMALE	LIVE PUPS
7326	7325	15	0	7	8 ^a	15	0	4	8	8	0	4	8
7327	7327	10	0	5	5 ^a	10	0	4	6	8	0	4	8
7328	7317	13	1	7	7	13	0	4	6	7	1	4	7
7329	7318	-	-	-	-	-	-	-	-	-	-	-	-
7330	7318	10	0	4	6 ^a	10	0	4	5	8	0	4	8
7331	7319	11	0	5	6 ^a	11	0	5	8	8	0	5	8
7332	7319	11	1	6	6	11	0	4	5	8	0	4	8
7333	7320	13	1	5	9	13	6	2	5	7	0	4	7
7334	7320	14	0	7	7	14	1	4	6	7	1	4	7
7335	7321	11	0	5	6 ^a	11	1	4	5	7	1	4	7
7336	7321	13	0	4	9 ^a	13	0	4	5	8	0	4	8
7337	7322	14	0	6	8	14	0	4	6	8	0	4	8
7338	7322	7	0	4	3	7	0	4	4	7	0	4	7
7339	7323	-	-	-	-	-	-	-	-	-	-	-	-
7340	7323	5	0	2	3	5	0	2	3	5	0	2	5
7341	7324	16	0	8	8	16	4	4	7	12	1	4	12
7342	7324	10	1	4	7	10	0	3	7	10	0	3	10
7343	7316	12	2	6	8	12	0	4	6	8	0	4	8
7344	7316	13	0	6	8 ^a	13	0	4	7	13	0	4	13
7345	7316	11	1	3	9	11	1	2	8	10	0	2	10
TOTAL		209	7	94	122	196	13	66	94	135	4	67	135
MEAN		12				11		73		8			8
SD		2.7				2.61							
SE		0.64				0.67							
N		11				11							

^a Pups mis-sexed.

TABLE II-E-23 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 11 (CONTINUED)

RESULTS OF F1b GENERATION - FIRST MATING (F2a)

DOSE - 750 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0		DAY 4				DAY 21				MEAN PUP WEIGHT (G)	
		LIVE PUPS	DEAD PUPS	SEX		LIVE PUPS	DEATHS DAY 1-4	SEX		LIVE PUPS	DEATHS DAY 5-21	LITTER REDUCED	
				MALE	FEMALE			MALE	FEMALE			MALE	FEMALE
7356	7355	13	0	6	7	7	0	6	7	13	0	4	4
7357	7354	14	0	6	8	6	0	6	8	14	0	4	4
7358	7347	15	0	10	5	7	0	10	5	15	0	4	4
7359	7353	13	0	5	8	6	0	5	8	13	0	4	4
7360	7348	-	-	-	-	-	-	-	-	-	-	-	-
7361	7361	13	0	6	7	6	0	6	7	13	1	4	4
7362	7349	-	-	-	-	-	-	-	-	-	-	-	-
7363	7364	6	0	4	2	8	0	4	2	6	0	4	2
7365	7350	-	-	-	-	-	-	-	-	-	-	-	-
7366	7351	14	0	8	6 ^a	5	0	8	6	14	0	4	4
7367	7352	10	0	4	6	6	0	4	6	10	1	3	5
7368	7352	9	0	3	6	7	0	3	6	9	0	3	5
7369	7353	7	0	5	2	6	0	5	2	7	0	5	2
7370	7353	-	-	-	-	-	-	-	-	-	-	-	-
7371	7371	11	0	8	3 ^a	7	0	7	4	11	0	4	4
7372	7354	13	0	8	5 ^a	5	0	8	5	13	0	4	4
7373	7373	12	0	6	6 ^a	6	3	7	2	12	0	6	2
7374	7346	12	0	5	7 ^a	6	0	6	6	12	0	4	4
7375													
TOTAL		162	0	84	78		3	83	76	159	2	57	52
MEAN		12				6				11			
SD		2.7				0.83				2.8			
SE		0.72				0.22				0.74			
N		14				14				11			

^aPup mls-saxad.

TABLE II-E-24

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 12

PUP OBSERVATIONS (F2a)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATIONS</u>
0	7301	0 4	ONE MALE - HEMATOMAS BOTH HIND LIMBS. ONE MALE - FOUND DEAD.
.80	7334	20	ONE MALE - FOUND DEAD; NO VISIBLE ABNORMALITIES.
	7335	4	ONE FEMALE - FOUND DEAD; ONE FEMALE - EMACIATED.
	7341	4	ONE FEMALE - MORIBUND; KILLED.
	7342	21	ONE MALE - SWOLLEN CEPHALIC REGION; NECROPSY SHOWED BRAIN WAS SMALL AND VERY SOFT, SURROUNDED BY LARGE QUANTITY OF FLUID.
750	7373	4	ONE FEMALE - FOUND DEAD.

TABLE II-E-25

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 13

PUP NECROPSY OBSERVATIONS (F2a)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>NUMBER OF PUPS</u>	<u>OBSERVATIONS</u>
0	7298	8	ALL PUPS APPEAR NORMAL.
	7299	8	ALL PUPS APPEAR NORMAL.
	7300	8	ALL PUPS APPEAR NORMAL.
	7305	8	ALL PUPS APPEAR NORMAL.
	7312	8	ALL PUPS APPEAR NORMAL.
	7314	8	ALL PUPS APPEAR NORMAL.
	7315	8	ALL PUPS APPEAR NORMAL.
80	7326	8	ALL PUPS APPEAR NORMAL.
	7332	8	ALL PUPS APPEAR NORMAL.
	7337	8	ALL PUPS APPEAR NORMAL.
	7340	5	ALL PUPS APPEAR NORMAL.
	7343	8	ALL PUPS APPEAR NORMAL.
	7345	8	ALL PUPS APPEAR NORMAL.
750	7357	8	ALL PUPS APPEAR NORMAL.
	7359	8	ALL PUPS APPEAR NORMAL.
	7361	7	ALL PUPS APPEAR NORMAL.
	7367	7	ALL PUPS APPEAR NORMAL.
	7374	8	ALL PUPS APPEAR NORMAL.

TABLE II-E-26

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 14

RESULTS OF F1b GENERATION - SECOND MATING (F2b)

DOSE - 0 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP				
		LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (G) MALE FEMALE	DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	LITTER REDUCED		DEATHS DAY 5-21	LIVE PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (G) MALE FEMALE	
									MALE	FEMALE					
7286	7288	8	0	3 5	8 7	1	7	2 5	2 5	0	7	2 5	2 5	56 55	
7297	7289	16	0	5 11	6 6	1	15	5 10	4 4	0	8	4 4	4 4	45 45	
7298	7291	15	0	4 11	7 6	0	15	4 11	4 4	0	8	4 4	4 4	49 47	
7299	7291	10	1	6 5	6 6	2	8	4 4	4 4	0	8	4 4	4 4	38 37	
7293	7300	13	0	8 5	6 5	0	13	8 5	4 4	0	8	4 4	4 4	47 45	
7301	7295	17	0	9 8	8 7	0	17	9 8	4 4	0	8	4 4	4 4	40 38	
7302	7286	14	0	4 10	6 6	0	14	4 10	4 4	0	8	4 4	4 4	49 51	
7303	7286	13	0	5 8	6 6	0	13	5 8	4 4	0	8	4 4	4 4	37 29	
7304	7289	14	2	6 10	5 5	1	13	4 9	4 4	0	8	4 4	4 4	45 45	
7305	7287	12	0	4 8	6 6	0	12	4 8	4 4	0	8	4 4	4 4	40 34	
7306	7293	13	0	8 5	6 6	0	13	8 5	4 4	0	8	4 4	3 ^a	45 41	
7307	7292	17	0	8 9	6 5	6	11	2 9	2 6	0	8	2 6	2 6	45 41	
7308	7294	12	0	5 7	6 6	0	12	5 7	4 4	0	8	4 4	4 4	32 32	
7309	7290	-	-	-	-	-	-	-	-	-	-	-	-	-	
7310	7287	13	0	7 6	6 5	1	12	6 6	4 4	0	8	4 4	4 4	45 41	
7311	7294	14	0	9 5	7 7	1	13	8 5	4 4	0	8	4 4	4 4	36 43	
7312	7292	12	0	6 6	8 7	0	12	6 6	4 4	1	7	4 4	3	55 55	
7313	7295	17	0	8 9	7 7	0	17	8 9	4 4	0	8	4 4	4 4	56 53	
7314	7288	16	0	9 7	6 5	0	16	9 7	4 4	1	7	3 4	3 4	50 48	
7315	7290	17	0	7 10	6 6	0	17	7 10	4 4	0	3	4 4	4 4	50 46	
TOTAL		263	3	121 145	6 6	13	250	108 142	72 79	2	149	72 77		45 43	
MEAN		14			0.84 0.75		13				8			6.8 7.4	
SD		2.5			0.19 0.17		2.7							1.6 1.7	
SE		0.57			19 19		0.63							19 19	
N		19					19								

^aMis-sexed.

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 14 (CONTINUED)

RESULTS OF F1b GENERATION

DOSE - 80 PPM

[illegible]

♂Pups mis-sexed.

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 14 (CONTINUED)

RESULTS OF F1b GENERATION - SECOND MATING (F2b)

DOSE - 750 PPM

FEMALE NUMBER		MALE NUMBER		DAY 0		DAY 4		DAY 21		MEAN PUP WEIGHT (G)		SEX		LITTER		DEATHS		LIVE		SEX		MEAN PUP WEIGHT (G)	
				LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (G) MALE FEMALE	DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	REDUCED MALE FEMALE	DEATHS DAY 5-21	LIVE PUPS	SEX MALE FEMALE	MALE FEMALE	MALE FEMALE	MALE FEMALE	MALE FEMALE					
7356	7351	13	1	6	8 ^a	7	7	0	0	13	7	6	4	4	0	0	8	58	56	4	4	4	4
7357	7353	13	0	5	8 ^a	7	7	0	0	13	7	7	4	4	0	0	8	54	50	4	4	4	4
7358	7354	16	0	7	9	6	6	0	0	16	6	9	4	4	0	0	8	55	54	4	4	4	4
7359	7346	14	0	4	10	6	5	0	0	14	6	10	4	4	0	0	8	47	50	4	4	4	4
7360	7353	12	0	5	7	6	6	0	0	12	6	7	4	4	0	0	8	38	34	4	4	4	4
7361	7348	14	0	6	8	6	6	0	0	14	6	8	4	4	0	0	8	42	45	4	4	4	4
7362	7354	12	1	8	5 ^a	5	5	0	0	12	5	3	5	3	1	1	7	42	38	4	3	4	3
7363	7349	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7364	7351	15	0	11	4	6	6	2	2	13	4	4	4	4	0	0	8	44	38	4	4	4	4
7365	7352	14	0	8	6 ^a	6	7	0	0	14	6	6	4	4	0	0	8	54	56	4	4	4	4
7366	7355	15	0	8	7 ^a	5	5	0	0	15	5	8	4	4	0	0	8	49	46	4	4	4	4
7367	7346	12	0	5	7	6	6	0	0	12	7	4	4	4	0	0	8	56	52	4	4	4	4
7368	7355	12	1	10	3	7	6	0	0	12	9	3	5	3	0	0	8	63	52	5	3	3	3
7369	7349	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7370	7350	15	0	8	7	6	6	0	0	15	8	7	4	4	0	0	8	53	54	4	4	4	4
7371	7352	11	2	7	6	7	6	0	0	11	6	5	4	4	0	0	8	51	47	4	4	4	4
7372	7347	15	0	6	9	6	6	0	0	15	6	9	4	4	0	0	8	53	49	4	4	4	4
7373	7347	13	0	5	8	6	6	0	0	13	5	8	4	4	0	0	8	49	46	4	4	4	4
7374	7348	14	0	10	4	6	5	14	14	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7375	7350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL		230	5	119	116			16		214	107	107	66	62	1	127	65	62					
MEAN		14				6	6			13						8			51	40			
SD		1.4				0.54	0.66			1.4									6.6	6.6			
SE		0.34				0.13	0.16			0.36									1.6	1.6			
N		17				17	17			16									16	16			

tops inched
downward.

TABLE II-E-27

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 15

PUP OBSERVATIONS (F2b)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATIONS</u>
0	7303	4	ONE FEMALE - HEMATOMA ON NOSE.
	7311	4	ONE MALE - FOUND DEAD.
80	7327	4	ONE MALE - FOUND DEAD.
	7336	2	ONE MALE - FOUND DEAD.
750	7361	0	ONE MALE - HEMATOMA ON NOSE.
	7374	4	ONE MALE - FOUND DEAD; NO OTHERS FOUND.

TABLE II-E-28

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 16

MEAN BODY WEIGHTS (G) OF F2b RATS

DOSE (PPM)	SEX		WEEK			
			4	9	11	20
0	M	MEAN	347	455	495	560
		SD	31	33	43	53
		SE	9.7	10	14	17
		N	10	10	10	10
80	M	MEAN	345	465	498	559
		SD	23	26	30	33
		SE	7.2	8.3	9.6	10
		N	10	10	10	10
750	M	MEAN	340	476	517	590
		SD	35	40	36	54
		SE	12	13	12	18
		N	9	9	9	9
0	F	MEAN	217	254	267	296
		SD	15	22	23	22
		SE	3.3	5.0	5.0	4.8
		N	20	20	20	20
80	F	MEAN	212	251	270	299
		SD	20	31	27	32
		SE	4.4	6.9	5.9	7.1
		N	20	20	20	20
750	F	MEAN	220	262	270	305
		SD	16	25	24	23
		SE	3.4	5.4	5.2	4.9
		N	21	21	21	21

TABLE II-E-29

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 17

MEAN FOOD CONSUMPTION (G) IN MALE F2b RATS

DOSE (PPM)	SEX		WEEK			
			4	9	11	20
0	M	MEAN	38	30	28	25
		SD	4.2	6.4	5.4	4.9
		SE	1.3	2.1	1.7	1.6
		N	10	9	10	9
80	M	MEAN	35	31	28	30
		SD	2.8	5.1	4.0	6.6
		SE	0.87	1.6	1.3	2.1
		N	10	10	10	10
750	M	MEAN	36	31	31	25
		SD	1.6	8.9	3.6	3.2
		SE	0.57	3.0	1.2	1.1
		N	8	9	9	9
0	F	MEAN	36	27	24	30
		SD	5.0	6.9	5.2	8.6
		SE	1.5	1.6	1.3	2.1
		N	11	18	17	17
80	F	MEAN	37	27	24	33
		SD	5.1	6.0	6.2	4.7
		SE	1.3	1.4	1.6	1.1
		N	15	19	15	17
750	F	MEAN	33	25	25	29
		SD	5.2	5.9	4.1	5.2
		SE	1.6	1.4	1.1	1.1
		N	11	18	15	21

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 18

RESULTS OF THIRD GENERATION - FIRST MATING (F3a)

DOSE - 0 PPM (CONTROL)

[illegible]

TABLE II-E-30 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 18 (CONTINUED)

RESULTS OF THIRD GENERATION - FIRST MATING (F3a)

DOSE - 80 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)	
		LIVE PUPS	DEAD PUPS	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 1-4	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 5-21	SEX MALE-FEMALE	LIVE PUPS	DEATHS DAY 5-21
1577A	1568A	12	0	4 8	4 8	0 0	4 8	12	0	4 4	4 4	41 41
1578A	1569A	4	0	1 3	1 3	0 0	1 3	4	0	1 3	1 3	57 57
1579A	1570A	12	0	7 5	7 5	0 0	7 5	12	0	4 4	4 4	41 41
1580A	1571A	13	0	8 5	8 5	2 2	6 5	11	0	4 4	4 4	46 46
1581A	1572A	14	0	9 5	9 5	0 0	9 5	14	0	4 4	4 4	47 43
1582A	1573A	16	0	10 6	10 6	0 0	10 6	16	0	4 4	4 4	46 44
1583A	1574A	13	0	5 8	5 8	0 0	5 8	13	0	4 4	4 4	45 45
1584A	1575A	15	0	8 7	8 7	0 0	8 7	15	0	4 4	4 4	45 41
1585A	1576A	13	0	7 6	7 6	0 0	7 6	13	0	4 4	4 4	48 45
1586A	1577A	14	0	7 7	7 7	6 6	1 7	8	0	2 0	2 0	46 48
1587A	1578A	11	0	6 5	6 5	0 0	6 5	11	0	4 4	4 4	47 46
1588A	1579A	16	0	9 7	9 7	0 0	9 7	16	0	4 4	4 4	49 47
1589A	1580A	13	0	5 8	5 8	0 0	5 8	13	0	4 4	4 4	41 45
1590A	1581A	15	0	8 7	8 7	0 0	8 7	15	0	4 4	4 4	45 41
1591A	1582A	13	0	7 6	7 6	0 0	7 6	13	0	4 4	4 4	48 45
1592A	1583A	14	0	7 7	7 7	6 6	1 7	8	0	2 0	2 0	46 48
1593A	1584A	2	0	2 0	2 0	0 0	2 0	2	0	0 0	0 0	47 46
1594A	1585A	11	0	6 5	6 5	0 0	6 5	11	0	4 4	4 4	47 46
1595A	1586A	16	0	9 7	9 7	0 0	9 7	16	0	4 4	4 4	49 47
1596A	1587A	13	0	5 8	5 8	0 0	5 8	13	0	4 4	4 4	41 45
1597A	1588A	15	0	8 7	8 7	0 0	8 7	15	0	4 4	4 4	45 41
1598A	1589A	13	0	7 6	7 6	0 0	7 6	13	0	4 4	4 4	48 45
1599A	1590A	14	0	7 7	7 7	6 6	1 7	8	0	2 0	2 0	46 48
1600A	1591A	2	0	2 0	2 0	0 0	2 0	2	0	0 0	0 0	47 46
1601A	1592A	11	0	6 5	6 5	0 0	6 5	11	0	4 4	4 4	47 46
1602A	1593A	16	0	9 7	9 7	0 0	9 7	16	0	4 4	4 4	49 47
TOTAL		195	1	103 93	103 93	8	90 88	187	0	61 57	118 7	46 42
MEAN		12				4.0		12				4.7
SD		3.9				0.99		4.0				1.2
SE		0.97				0.32		0.99				1.1
N		16				16		16				16

TABLE II-E-30 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 18 (CONTINUED)

RESULTS OF THIRD GENERATION - FIRST MATING (F3a)

DOSE - 750 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21								
		LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (g)		DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	LITTER REDUCED		DEATHS DAY 5-12	LIVE PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (g)	
					MALE	FEMALE				MALE	FEMALE				MALE	FEMALE
1607A	1597A	-	1	10 6	6 5	0	15	9 6	-	4 4	-	-	8	4 4	-	44 43
1608A	1598A	18	0	7 11	7 6	1	17	7 10	0	4 4	0	0	8	4 4	4 4	45 45
1610A	1599A	12	0	6 6	8 8	0	12	6 6	0	4 4	0	0	8	4 4	4 4	55 47
1611A	1599A	13	0	4 9	6 6	0	13	4 9	0	4 4	0	0	8	4 4	4 4	54 50
1612A	1600A	12	0	7 5	8 7	1	11	7 4	0	4 4	0	0	8	4 4	4 4	52 49
1613A	1600A	16	0	9 7	6 6	0	16	9 7	0	4 4	0	0	8	4 4	4 4	46 42
1614A	1601A	13	0	5 8	6 6	0	13	5 8	0	4 4	0	0	8	4 4	4 4	37 34
1615A	1601A	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
1616A	1617A	15	1	7 9	6 6	0	15	7 8	0	4 4	0	0	8	4 4	4 4	56 55
1617A	1618A	14	0	9 5	7 7	0	14	9 5	0	4 4	0	0	8	4 4	4 4	53 50
1618A	1619A	12	0	6 6	7 6	0	12	6 6	0	4 4	0	0	8	4 4	4 4	50 46
1619A	1620A	12	0	9 3	6 6	0	12	9 3	0	5 3	0	0	8	5 3	48 48	
1620A	1621A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1621A	1622A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1622A	1604A	10	0	7 3	6 6	0	10	7 3	0	5 3	3	3	5	4 1	38 37	
1623A	1624A	14	0	7 7	7 7	0	14	7 7	0	4 4	0	0	8	4 4	43 39	
1624A	1605A	14	0	7 7	6 5	0	14	7 7	0	4 4	0	0	8	4 4	43 40	
1625A	1626A	14	0	8 6	8 7	1	13	7 6	0	4 4	0	0	8	4 4	53 49	
1626A	1626A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TOTAL	204	2	108	98	7 6	3	201	106 95	62 58	3	117	61 56	48 45			
MEAN	14	2.0	0.51	15	0.82 0.80	1.88	0.49	15 15	0.21 0.21	15 15	0.21 0.21	1.5 1.5	6.1 5.7			
SD	2.0	0.51	15	0.82 0.80	1.88	0.49	15 15	0.21 0.21	15 15	0.21 0.21	1.5 1.5	6.1 5.7	6.1 5.7			
SE	0.51	15	0.82 0.80	1.88	0.49	15 15	0.21 0.21	15 15	0.21 0.21	15 15	0.21 0.21	1.5 1.5	6.1 5.7			
N	15	0.51	15	0.82 0.80	1.88	0.49	15 15	0.21 0.21	15 15	0.21 0.21	1.5 1.5	6.1 5.7	6.1 5.7			

a Not bred; male No. 1606A mis-sexed.

TABLE II-E-31

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 19

PUP OBSERVATIONS (F3a)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATIONS</u>
0	1547	11	THREE MALES - FOUND DEAD; TWO FEMALES - FOUND DEAD; ONE PUP CANNIBALIZED (SEX NOT DETERMINED).
		12	ONE MALE - FOUND DEAD.
80	1595	0	ONE FEMALE - PART OF TAIL APPARENTLY BITTEN OFF.
		4	ONE FEMALE - SHORTENED TAIL.
750	1609	3	ONE FEMALE - FOUND DEAD.
	1625	4	ONE MALE - FOUND DEAD.

TABLE II-E-32

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 20

PUP NECROPSY OBSERVATIONS (F3a)

DOSE (PPM)	FEMALE NUMBER	NUMBER OF PUPS	OBSERVATIONS
0	1555A	8	ALL TISSUES APPEAR NORMAL.
	1560A	8	ALL TISSUES APPEAR NORMAL.
	1563A	8	ALL TISSUES APPEAR NORMAL.
	1566A	8	ALL TISSUES APPEAR NORMAL.
80	1577A	8	ALL TISSUES APPEAR NORMAL.
	1582A	8	ALL TISSUES APPEAR NORMAL.
	1583A	8	ALL TISSUES APPEAR NORMAL.
	1589A	8	ALL TISSUES APPEAR NORMAL.
	1594A	2	ALL TISSUES APPEAR NORMAL.
750	1609A	8	ALL TISSUES APPEAR NORMAL.
	1613A	8	ALL TISSUES APPEAR NORMAL.
	1619A	8	THREE PUPS - MOTTLED THYMUS.
	1624A	8	ALL TISSUES APPEAR NORMAL.
	1625A	8	ALL TISSUES APPEAR NORMAL.

TABLE II-E-33

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 21

RESULTS OF F2b GENERATION - SECOND MATING (F3b)

DOSE - 0 PPM (CONTROL)

FEMALE NUMBER	MALE NUMBER	DAY 0		DEAD PUPS	SEX		MEAN PUP WEIGHT (G)		DAY 4		SEX		LITTER		DAY 21		MEAN PUP WEIGHT (G)		
		LIVE PUPS	PUPS		MALE	FEMALE	MALE	FEMALE	DEATHS DAY 1-4	LIVE PUPS	MALE	FEMALE	REDUCED	MALE	FEMALE	DEATHS DAY 5-21	LIVE PUPS	MALE	FEMALE
1547A	1538A	15	0	0	5	10	6	6	0	15	8	7 ^a	4	4	4	0	8	4	4
1548A	1537A	9	0	0	5	4	8	7	0	9	5	4	4	4	4	0	8	5	3 ^a
1549A	1536A	7	0	0	6	1	7	7	0	7	5	2 ^a	5	2	2	0	7	5	2
1550A	1535A	9	1	1	4	6	5	5	0	9	6	3 ^a	5	3	3	0	8	4	4
1546A	1546A	12	0	0	6	6	6	6	0	12	6	6	4	4	4	0	8	4	4
1551A	1545A	10	1	1	5	6	7	7	0	10	5	5	4	4	4	0	8	4	4
1552A	1545A	13	3	3	5	9	6	6	0	13	5	8	4	4	4	0	8	4	4
1553A	1544A	17	0	0	7	10	6	6	1	16	7	9	4	4	4	0	8	4	4
1554A	1544A	14	0	0	5	9	6	5	0	14	5	9	4	4	4	0	8	4	4
1555A	1543A	-	0	0	-	-	-	-	0	-	-	-	-	-	-	0	-	-	-
1556A	1543A	13	0	0	5	8	6	6	0	13	5	8	4	4	4	0	8	4	4
1557A	1542A	11	1	1	4	8	7	7	0	11	3	8	3	5	5	0	8	3	5
1558A	1542A	13	0	0	6	7	7	7	0	13	6	7	4	4	4	0	8	4	4
1559A	1541A	16	0	0	4	12	7	6	2	14	4	10	4	4	4	1	7	4	3
1560A	1541A	13	0	0	9	4	6	6	0	13	9	4	4	4	4	0	8	4	4
1561A	1562A	15	0	0	4	11	5	6	1	14	4	10	4	4	4	0	8	4	4
1562A	1540A	-	0	0	-	-	-	-	0	-	-	-	-	-	-	0	-	-	-
1563A	1564A	-	0	0	-	-	-	-	0	-	-	-	-	-	-	0	-	-	-
1564A	1539A	14	0	0	6	8	7	6	0	14	6	8	4	4	4	0	8	4	4
1565A	1566A	10	0	0	7	3	7	6	0	10	7	3	5	3	3	0	8	5	3
TOTAL		211	4	4	93	122	6	6	4	207	96	111	70	65	64	1	134	70	64
MEAN		12					0.79	0.64		12							8		
SD		2.7					0.19	0.15		2.46									
SE		0.66								0.60									
N		17								17									

^apups m/s-sexed.

TABLE II-E-33 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 21 (CONTINUED)

RESULTS OF F2b GENERATION - SECOND MATING (F3b)

DOSE - 80 PPM

FEMALE NUMBER	MALE NUMBER	DAY 0			DAY 4			DAY 21			MEAN PUP WEIGHT (G)			
		LIVE PUPS	DEAD PUPS	SEX MALE FEMALE	MEAN PUP WEIGHT (G) MALE FEMALE	DEATHS DAY 1-4	LIVE PUPS	SEX MALE FEMALE	LITTER REDUCED MALE FEMALE	DEATHS DAY 5-21	LIVE PUPS	SEX MALE FEMALE	MALE	FEMALE
1577A	1576A	11	0	5 6	6 6	0	11	5 6	4 4	0	8	4 4	33	31
1570A	1570A	13	0	7 7	6 6	0	13	7 6	4 4	0	8	4 4	57	50
1579A	1567A	11	0	8 3	9 8	0	11	8 3	5 3	0	8	5 3	37	31
1580A	1580A	9	1	4 6	7 6	0	9	3 6	3 5	0	8	3 5	32	31
1575A	1575A	15	0	7 8	6 6	0	15	7 8	4 4	0	8	3 5 ^a	32	27
1581A	1581A	15	0	7 8	6 6	0	15	7 8	4 4	0	8	3 5 ^a	32	27
1582A	1582A	15	0	12 3	9 8	0	15	12 3	5 3	0	8	4 4 ^a	55	55
1583A	1574A	15	0	6 9	6 6	0	15	6 9	4 4	0	8	4 4	55	53
1584A	1584A	15	1	6 10	6 6	0	15	5 10	4 4	0	8	4 4	46	44
1585A	1573A	-	-	-	-	0	-	-	-	-	-	-	-	-
1586A	1586A	13	4	10 7	6 5	0	13	8 5	4 4	0	8	4 4	28	17
1587A	1572A	13	0	7 6	7 7	0	13	7 6	4 4	1	7	3 4	37	32
1588A	1588A	-	-	-	-	0	-	-	-	-	-	-	-	-
1589A	1571A	9	0	5 4	7 7	0	9	5 4	4 4	0	8	4 4	32	34
1590A	1570A	-	-	-	-	0	-	-	-	-	-	-	-	-
1591A	1591A	15	0	9 6	7 7	0	15	9 6	4 4	0	8	4 4	41	40
1592A	1569A	15	0	10 5	6 6	0	15	10 5	4 4	0	8	4 4	46	50
1593A	1593A	-	-	-	-	0	-	-	-	-	-	-	-	-
1568A	1568A	8	1	1 8	6 5	0	8	2 6 ^a	2 6	0	8	2 6	53	51
1594A	1594A	14	0	4 10	7 6	0	14	5 9 ^a	4 4	0	8	4 4	56	51
1595A	1595A	15	0	6 9	7 6	0	15	6 9	4 4	0	8	4 4	57	53
1596A	1571A	15	0	6 9	7 6	0	15	6 9	4 4	0	8	4 4	57	53
TOTAL		206	7	107 106		0	206	104 101	63 67	1	127	60 67	44	41
MEAN		13			7 6		13				11		11	12
SD		2.6			0.98 0.87		2.6						2.7	2.9
SE		0.63			0.26 0.22		0.63						1.6	1.6
		16			1.6 1.6		16							

*Pups mated.

TABLE II-F-33 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 21 (CONTINUED)

RESULTS OF F2b GENERATION - SECOND MATING (F3b)

DOSE - 750 PPM

FEMALE NUMBER	MALE NUMBER	DEAD PUPS	SEX		LIVE PUPS		SEX		LITTER REDUCED		DEATHS DAY 5-21		SEX		MEAN PUP WEIGHT (G)	
			MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
1607A	Not bred	0	3	2	8	7	0	1	3	2	1	4	3	1	47	40
1608A	1605A	0	11	5	7	6	1	15	11	4	0	8	4	4	31	32
1609A	1604A	0	4	6	8	8	0	10	4	4	0	8	4	4	43	40
1610A	1604A	0	7	7	7	7	0	14	7	7	0	8	4	4	63	58
1611A	1603A	0	8	4	5	5	0	12	6	6 ^a	1	7	4	3	28	30
1612A	1602A	0	8	5	6	6	0	13	8	5	0	8	4	4	28	30
1613A	1602A	0	5	8	7	6	1	12	5	7	0	8	4	4	32	26
1614A	1601A	0	6	8	7	7	0	14	6	8	0	8	4	4	41	46
1615A	1603A	0	5	6	7	7	0	11	6	5 ^a	0	8	4	4	47	48
1616A	1600A	2	7	8	7	7	0	13	6	7	1	7	3	4	47	40
1617A	1599A	1	6	7	7	6	0	12	5	7	0	8	4	4	62	57
1618A	1599A	1	4	10	8	7	0	14	4	10	0	8	4	4	35	33
1619A	1596A	0	7	8	6	5	0	15	7	8	0	8	4	4	37	41
1620A	1597A	0	6	10	6	6	1	15	6	9	0	8	4	4	53	45
1621A	1597A	0	6	10	6	6	1	15	6	9	0	8	4	4	53	45
1622A	1597A	0	6	10	6	6	1	15	6	9	0	8	4	4	53	45
1623A	1597A	0	6	10	6	6	1	15	6	9	0	8	4	4	53	45
1624A	1597A	0	6	10	6	6	1	15	6	9	0	8	4	4	53	45
1625A	Not bred	0	6	4	7	7	0	10	5	5	0	8	4	4	43	44
1626A	1601A	0	6	4	7	7	0	10	5	5	0	8	4	4	43	44
TOTAL	1601A	3	93	98	7	6	3	185	89	96	3	114	57	57	43	41
MEAN	1601A	13			0.83	0.83		12				8			11	9.5
SD	1601A	2.8			0.21	0.21		0.68							2.9	2.4
SE	1601A	0.72			15	15		15							15	15
N	1601A	15														

^a Pups mis-sexed.

TABLE II-E-34

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 22

PUP OBSERVATIONS (F3b)

<u>DOSE (PPM)</u>	<u>FEMALE NUMBER</u>	<u>DAY OF LACTATION</u>	<u>OBSERVATIONS</u>
0	1550A	4	ONE FEMALE - SCAB ON NOSE.
	1554A	4	ONE FEMALE - FOUND DEAD.
	1559A	4	ONE MALE - BENT TAIL.
	1560A	4	TWO FEMALES - CANNIBALIZED.
	1562A	4	ONE FEMALE - CANNIBALIZED.
80	1586A	4	ONE FEMALE - CANNIBALIZED
	1587A	4	ONE MALE - SCAB ON NOSE.
750	1609A	4	ONE FEMALE - CANNIBALIZED.
	1614A	4	ONE FEMALE - CANNIBALIZED.
	1628A	4	ONE FEMALE - CANNIBALIZED.

TABLE II-E-35

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 23

PUP NECROPSY OBSERVATIONS (F3b)

DOSE (PPM)	FEMALE NUMBER	NUMBER OF PUPS	OBSERVATIONS
0	1550A	8	ALL TISSUES APPEAR NORMAL.
	1552A	8	ALL TISSUES APPEAR NORMAL.
	1555A	8	ALL TISSUES APPEAR NORMAL.
	1557A	8	ALL TISSUES APPEAR NORMAL.
	1558A	8	ONE FEMALE - MOTTLED KIDNEYS.
	1560A	7	ALL TISSUES APPEAR NORMAL.
	1566A	8	ALL TISSUES APPEAR NORMAL.
80	1578A	8	ALL TISSUES APPEAR NORMAL.
	1579A	8	ALL TISSUES APPEAR NORMAL.
	1580A	8	ALL TISSUES APPEAR NORMAL.
	1582A	8	ALL TISSUES APPEAR NORMAL.
	1586A	8	ALL TISSUES APPEAR NORMAL.
	1587A	5	ALL TISSUES APPEAR NORMAL.
	1595A	8	ALL TISSUES APPEAR NORMAL.
750	1608A	4	ALL TISSUES APPEAR NORMAL.
	1613A	8	ALL TISSUES APPEAR NORMAL.
	1616A	8	ALL TISSUES APPEAR NORMAL.
	1617A	8	ALL TISSUES APPEAR NORMAL.
	1619A	8	ALL TISSUES APPEAR NORMAL.
	1622A	8	ALL TISSUES APPEAR NORMAL.
	1623A	8	ALL TISSUES APPEAR NORMAL.

TABLE II-E-36

LITTON BIONETICS, INC.
PROJECT NO. 10734-07

TABLE 24

ADULT NECROPSY OBSERVATIONS (F2b)

<u>DOSE (PPM)</u>	<u>SEX</u>	<u>ANIMAL NUMBER</u>	<u>OBSERVATIONS</u>
0	M F	1561A	NO VISIBLE ABNORMALITIES. HARD MASS (1.5 X 1.5 CM) MAMMARY REGION, RIGHT LATERAL THORAX.
80	M F	1581A	NO VISIBLE ABNORMALITIES. MOTTLED KIDNEYS.
750	M F F	1616A 1626A	NO VISIBLE ABNORMALITIES. MOTTLED KIDNEYS. MOTTLED KIDNEYS.

SPONSOR: Environmental Protection Department
US Army Medical Bioengineering Research and
Development Laboratory

MATERIAL: Dicyclopentadiene (DCPD)

SUBJECT: FINAL REPORT
Analysis of Diet Formulations
LBI Project No. 10734-7

1. OBJECTIVE

The objective of this study was to analyze DCPD in animal chow with regards to stability and formulation content in diet.

2. MATERIAL AND EXPERIMENTAL DESIGN

The analysis of the/dosed feed was performed by the following chromatographic method.

Scope

This mechod describes the analytical procedure for the determination of DCPD in dosed feed used by Litton Bionetics, Inc. (LBI) during the course of the study.

Principle

A five gram feed subsample is extracted with 20 ml of diethyl ether by shaking for 15 min in an automated shaker. The extract is clarified by centrifugation for 10 min at 1350 rpm. The extracts are analyzed with a Varian 2100 gas chromatograph equipped with flame ionization detectors.

The DCPD content is calculated from a calibration curve obtained by GLC analysis of reference solutions of DCPD in ether. Control and spiked control feed samples are analyzed concurrently to correct for possible feed background and compound recovery.

Equipment and Supplies

Graduated conical Falcon tubes, 50 ml, with positive seal caps (available from Becton, Dickinson and Company, Oxnard, CA, 93030; stock number H8292-209811).

Volumetric glassware - 1, 4, 5, and 10 ml pipettes; 50 and 100 ml flasks.

Graduated cylinder - 25 ml capacity.

Graduated glass centrifuge tubes, 15 ml, with ground glass stoppers.

Mechanical shaker.

Centrifuge.

Analytical laboratory balance (accurate to 0.01 mg).

Top-loading laboratory balance (accurate to 0.01g).

Gas-liquid chromatograph - Varian 2100, equipped with a 1.8 m x 2 mm I.D. glass column packed with 10% FFAP on 80/100 mesh Supelcoport, flame ionization detectors.

Diethyl ether (Burdick and Jackson).

Dicyclopentadiene.

Preparation of Standard

Prepare a stock standard solution of DCPD by dissolving 50 mg of DCPD in 50 ml of Acetone.

Take a 5 ml aliquot and dilute to 100 ml with diethyl ether in a volumetric flask. This solution has a concentration of 0.05 mg/ml.

Prepare a standard curve by injecting 1, 2 and 3 μ l of the standard solution into a Varian 2100 gas chromatograph with the following parameters:

Column temperature:	60°C
Injector temperature:	225°C
FID temperature:	250°C
Chart:	6 min/inch
Carrier gas flow:	40 cc/min nitrogen
Attn.:	8×10^{-11}

Procedure:

Weigh a 5 g sample of the dosed feed to the nearest 0.01 g in a Falcon tube.

Extract the sample with 20 ml of diethyl ether by mixing for 15 min in a mechanical shaker; followed by centrifugation at 1300 rpm for 10 min.

Dilute the high-dose level (750 ppm) in a 15 ml graduated centrifuge tube by adding a 1 ml aliquot to 4 ml of diethyl ether.

Repeat above procedure using undosed animal feed of the same lot used for the preparation of the dosed feed. This extract will be used as a negative control to assure that there are no interfering peaks contributed by the feed itself.

Repeat above procedure using undosed animal feed of the same lot which has been spiked in the laboratory with DCPD at corresponding dose levels.

Quantitate the amount of DCPD in solution by comparing to the calibration curve prepared above.

Calculations

Calculate the ppm of DCPD in the dosed feed or spiked (recovery) sample as follows:

To determine mg of sample injected:

$$\frac{5g \text{ feed}}{20 \text{ ml ether}} = \frac{250 \text{ mg feed}}{1.0 \text{ ml ether}}$$

$$\frac{250 \text{ mg feed}}{1.0 \text{ ml ether}} \times \text{Dilution Factor} = \frac{(x) \text{ mg feed}}{1.0 \text{ ml ether}}$$

$$\begin{aligned} \text{Dilution Factor} &= 1 \text{ for 80 ppm level} \\ &= 0.2 \text{ for 750 ppm level} \end{aligned}$$

$$\frac{(x) \text{ mg feed}}{1.0 \text{ ml ether}} \times \frac{\mu\text{l sample}}{1000} = \text{mg of feed injected}$$

Calculate the intercept and slope from standard curve as determined by linear regression correlation.

$$\frac{\text{Peak Response (peak height)} - \text{intercept}}{\text{slope}} = \text{ng of DCPD injected}$$

To determine ppm:

$$\frac{\text{ng of DCPD found}}{\text{mg of feed injected}} = \text{ppm}$$

Determine method recovery from spiked samples as follows:

$$\text{percent recovery} = \frac{\text{ppm found} \times 100}{\text{ppm added}}$$

Correct the result of the dosed feed sample for method recovery of its corresponding spiked sample.

$$\text{corrected ppm} = \frac{\text{sample ppm} \times 100}{\text{percent recovery}}$$

3. RESULTS

Stability Analysis

Samples were analyzed the day the mix was received by the analytical laboratory. This corresponded to Day 1 of the stability study when Day 0 is considered the mix date. Two aliquots of feed were removed from each diet level of samples 0433, 0434 and 0435 and stored at ambient conditions. One aliquot was stored in a closed container, while the other was stored in an open container.

The two aliquots were analyzed 9 days later (day 10) by the standard method. Results of the analysis are indicated in Table 1.

In the closed containers, the concentration dropped 27.6% for the 80 ppm level and 30.8% for the 750 ppm level.

In the open containers, after 10 days no DCPD could be detected in either high- or low-dose samples.

DCPD is a liquid which at ambient temperature tends to volatilize from the feed mixture. This may account for the results noted in the open container stability study. In the closed container the DCPD may volatilize as much as the head space in the storage container will permit. At the saturation point, an equilibrium between the vapors and liquid is achieved and the concentration of DCPD in feed remains constant. This appears to be at 70% of the theoretical concentration.

Weekly Diet Analysis

DCPD samples were analyzed on a weekly basis by the method previously described. Samples were received by the analytical laboratory and stored at ambient conditions for the first five feed mixtures. Thereafter, samples were stored in the freezer. This action was required due to the volatile nature of the compound. Results of the analysis are indicated in Table 2.

For the 80 ppm level, the average value of the course of the study was 69.3 ± 8.2 ppm. This corresponds to $86.6 \pm 10.3\%$ of the theoretical concentration.

For the 750 ppm level, the average value obtained was 693 ± 68.0 ppm, which corresponds to $92.4 \pm 9.1\%$ of the theoretical value.

The values for the rat reproduction study was, on the average, approximately 10% below the theoretical value. This fact may be attributed to the volatile nature of DCPD, and the time between mixing and analysis. During the latter part of the study, the interval between the analysis and the mixing was decreased as much as possible. An improvement in the analytical values resulted.

LITTON BIONETICS, INC.
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SUBMITTED BY:

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14 May 1979
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PART II - SECTION F
THREE-MONTH SUBCHRONIC TOXICITY IN DOGS

DCPD

LBI PROJECT NO. 10734-09

SUMMARY

The test material, Dicyclopentadiene, was administered by incorporation into the diet at concentrations of 100, 300, and 1000 ppm to Beagle dogs for 13 weeks. The animals were observed daily for general condition and behavior. Clinical pathological evaluations, including analysis of the clinical chemical constituents of serum, urine and hemograms, were performed at approximately monthly intervals. Tissues from the control and high dose dogs were compared histopathologically for differences. Based on the results obtained using these criteria it was concluded that treatment produced no significant toxicity with the possible exception of minor indications of intestinal distress expressed as vomiting and soft stools among dogs of the treated groups, especially the highest dose (1000 ppm).

OBJECTIVE

The objective of this study was to evaluate and characterize the toxicity of the test material by incorporation in the diet to dogs for 13 weeks.

MATERIAL

Refer to Part II - Section A.

EXPERIMENTAL DESIGN

Thirty-four purebred beagle dogs (approximately five months of age) were received from Laboratory Research Enterprises, Inc. (LRE), Kalamazoo, Michigan, and maintained for 4 months in the Falls Church facility of the Department of Toxicology. The planned initiation of the study was delayed under the direction of the LBI Department of Safety and Health because of a possible hazard to personnel. The dogs were transferred to the Rockville facility of the Department of Toxicology and acclimated for 4 weeks. The animals were individually housed in stainless steel cages in temperature-controlled quarters under artificial illumination controlled to provide a 12-hour light cycle. Purina Dog Chow and water were provided ad libitum.

Prior to initiation of the study, all dogs were given a preliminary health screening which included clinical biochemical, hematological, ophthalmological and parasitological testing. Examination showed that 17 out of 34 dogs had parasites. In 16 dogs, Giardia canis cysts were found. Isopora species oocysts were found in two dogs and Trichomonas species in one dog. Since the pathogenicity of the mentioned parasites was questionable, treatment was not initiated. All dogs had been immunized by LPF against distemper, hepatitis, leptospirosis and rabies prior to receipt.

Thirty-two dogs were selected for use in the study. The dogs had been identified by tattoo at LRE and were given LBI dog numbers. Dogs were randomly selected and placed into groups as noted below.

<u>Group Number</u>	<u>Number of Animals</u>		<u>Dose (ppm)</u>
	<u>Males</u>	<u>Females</u>	
1	4	4	0
2	4	4	100
3	4	4	300
4	4	4	1000

Initial clinical pathological determinations did not reveal any abnormalities. Following these determinations, two dogs (Nos. 00410 and 00428) were eliminated from the study. The remaining dogs were chosen for the study and assigned to groups as detailed in Text Table A. Date of first dose was May 10, 1978.

Dietary concentrations were selected by a representative of the sponsor. The feed and test material were mixed weekly. A premix was prepared in corn oil, manually mixed with the appropriate amount of test material and blended with the dog meal for 20 minutes in a blender. A sample of each weekly formulation was sent to the LBI Analytical Laboratory for analysis with respect to correctness of formulation.

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LITTON BIONETICS, INC.
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TABLE A

GROUP ASSIGNMENT

<u>GROUP NUMBER</u>	<u>DOSE LEVEL (PPM)</u>	<u>SEX</u>	<u>L.R.E. TATTOO NUMBER</u>	<u>LBI NUMBER</u>
1	0	M	PK 77	408
		M	DI 77	411
		M	QS 77	417
		M	MK 77	418
		F	GZ 77	425
		F	EI 77	426
		F	LB 77	434
		F	EZ 77	435
2	100	M	LS 77	405
		M	NW 77	406
		M	GB 77	407
		M	LI 77	415
		F	FJ 77	422
		F	LL 77	429
		F	OH 77	430
		F	IR 77	431
3	300	M	MM 77	404
		M	LT 77	409
		M	OL 77	412
		M	NY 77	414
		F	OV 77	421
		F	FO 77	424
		F	AR 77	427
		F	LO 77	433
4	1000	M	MB 77	413
		M	PI 77	416
		M	OJ 77	419
		M	KK 77	420
		F	JL 77	423
		F	HO 77	432
		F	IT 77	436
		F	FF 77	437

EXPERIMENTAL DESIGN (Continued)

The dogs were observed daily for general appearance, behavior, food consumption and fecal consistency. Body weights were recorded weekly on each animal. Initially and at 4, 8 and 13 weeks, blood was collected for the following pathological determinations. The dogs were fasted overnight prior to blood collection.

Hematology

hemoglobin
erythrocytes
leukocytes
differential count
packed cell volume

Blood Chemistry

glucose	alkaline phosphatase
calcium	total protein
urea nitrogen	albumin
serum glutamic-pyruvic transaminase	cholesterol
serum glutamic-oxaloacetic transaminase	lactic dehydrogenase
uric acid	phosphorus
potassium ^a	bilirubin
	sodium ^a
	chloride ^a

^aPerformed initially, only.

Urinalysis was performed on all animals initially and at 8 and 13 weeks. The dogs were fasted overnight prior to urine collection for determination of the following.

specific gravity	ketones
pH	occult blood
color	bilirubin
sugar	microscopic examination of sediment
albumin	

Ophthalmologic examinations were performed initially and before termination of the study. These evaluations were conducted by James M. Clinton, V.M.D., Consulting Veterinary Ophthalmologist.

After 94 to 97 days of treatment, the dogs were killed. Each animal was weighed and subjected to a complete necropsy. Appropriate samples of each of the following organs and tissues were preserved in 10% neutral buffered formalin.

brain ^a	stomach
pituitary	small intestines
spinal cord	large intestines
eye	testes with epididymis ^a

EXPERIMENTAL DESIGN (Continued)

thyroid^a
pancreas
lung
heart^a
rib junction
gallbladder
liver^a
spleen^a
kidneys^a
adrenal glands^a

prostate
ovary^a
uterus
bone marrow
skeletal muscle and nerve
urinary bladder
mammary gland
mesenteric lymph node
any unusual lesions

^aOrgan weights taken.

The tissues from dogs of the control and high level groups were evaluated pathologically by G.A. Parker, D.V.M.

Statistical analysis was performed using Dunnett's t-test to determine differences between treated and control means of the same sex. A probability value of <0.05 was used as a basis of statistical inference.

RESULTS

All dogs survived the entire duration of the study. The clinical signs (observations) noted during the course of the study have been compiled and presented in Appendix Table 1. Review of these data suggested no remarkable difference between treated and control dogs with the possible exception of a slightly higher frequency of vomiting and soft stools among the treated dogs, especially those of the high level (1000 ppm). These signs were occasionally observed among the control dogs as well.

The body weights obtained during the course of the study have been tabulated in Appendix Table 2, and summarized in Text Table B. The mean body weights of treated and control groups were similar statistically and review of the individual body weights did not suggest any progressive effect of treatment.

The food consumption presented as daily food intake (g/day) has been tabulated in Appendix Table 3, and summarized in Text Table C. Weeks at which the food intake among the treated and control groups were statistically different were limited and were not judged to suggest a compound-related effect.

The results of the hemograms obtained initially and at 4, 8 and 13 weeks (termination) have been summarized in Text Table D. The individual data at these intervals have been detailed in Appendix Table 4. No dose-related effect was evident.

Results of the clinical chemistry determinations obtained initially and at Weeks 4, 8 and 13 (termination) of the study have been summarized in Text Table E. The individual data for these intervals have been detailed in Appendix Table 5. Statistically significant differences between control and treated groups were minimal and were not suggestive of a meaningful compound-related effect. The apparent increase in serum glucose at the 1000 ppm level for males at termination was not judged to be of real importance inasmuch as both male dogs on which data were available were well within normal limits.

The individual results of the urinalysis have been detailed in Appendix Table 6. Review of these data did not suggest any dose-related effects.

The individual organ weights and organ-to-body weight ratios have been detailed in Appendix Tables 7 and 8, respectively and summarized in Text Tables F and G, respectively. Review suggested a decreased thyroid size; however, statistical analysis did not indicate the difference was significant.

Analysis results of the diet formulations have been included in the Appendix. The diet proportions analyzed at means of 98, 101 and 99% of the theoretical concentrations for the 100, 300 and 1000 ppm dietary concentrations. Since the test material tended to vaporize, the actual intake may have been somewhat less than the intended dietary levels would have suggested.

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LITTON BIONETICS, INC.
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TABLE B

MEAN BODY WEIGHTS (KG)

MALESDOSE LEVEL
(PPM)

WEEK	0	1	2	3	4	5	6	7	8	9	10	11	12	13	TERMINATION
CONTROL (0)	10.8	10.7	10.6	10.8	11.0	10.6	10.8	10.8	10.7	11.1	11.2	11.1	11.6	11.0	10.9
100	12.1	11.8	11.8	12.0	12.2	11.8	12.0	12.2	12.2	12.3	12.3	12.5	12.4	11.9	11.6
300	10.0	9.8	9.7	9.9	10.1	9.8	10.0	10.2	10.1	10.3	10.4	10.3	10.4	9.9	9.8
1000	10.8	10.5	10.8	10.6	10.9	10.6	10.7	10.7	10.5	10.6	10.6	10.7	10.9	10.4	10.4
<u>FEMALES</u>															
CONTROL (0)	8.1	7.7	7.6	7.8	7.9	7.5	7.6	7.6	7.6	7.8	8.0	7.9	8.0	7.6	7.5
100	9.0	8.8	8.8	8.8	9.0	8.6	8.9	9.0	9.0	9.3	9.2	9.1	9.3	9.1	8.8
300	8.8	8.8	8.7	8.9	9.3	9.1	9.0	9.1	9.0	9.4	9.4	9.5	9.3	8.9	8.8
1000	8.3	8.1	8.0	8.1	8.9	8.1	8.2	8.4	8.3	8.3	8.3	8.3	8.4	7.9	7.9

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LITTON BIONETICS, INC.
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TABLE C

MEAN DAILY FOOD INTAKE (G)

MALESDOSE LEVEL
(PPM)

WEEK

	1	2	3	4	5	6	7	8	9	10	11	12	13
CONTROL (0)	346.4	324.3	366.5	278.0	344.1	297.2	297.4	367.3	353.0	345.1	296.1	363.1	315.2
100	330.0	353.8	319.9	361.3	363.8	349.2	329.6	293.4	343.1	316.4	310.1	309.7	319.8
300	265.6	274.9	291.6	312.8	284.9	272.7	303.9	241.4	263.3	299.7	304.5	262.5*	301.8
1000	328.9	332.6	292.8	362.9	271.1	282.2	291.8	255.4	290.7	208.5	289.0	374.2	243.1

FEMALES

CONTROL (0)	262.8	242.2	267.7	246.7	345.3	^a	304.9	268.5	281.5	287.6	350.2	251.8	268.6
100	244.6	230.5	266.8	249.9	245.6	259.3	264.1	241.9	243.2	231.5	243.0	220.6	200.7
300	269.1	257.3	295.7	238.1	238.6	229.9	298.1	227.2	316.6	275.2	251.6	252.9	260.3
1000	223.8	219.5	246.1	208.2	222.8	232.6	252.1	241.4	229.0	199.7	222.7*	263.1	245.5

^aAll spilled food.

* p<0.05 as compared to controls: Dunnett's t-test.

TABLE II-F-40

LITTON BIONETICS, INC.
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TABLE D

CLINICAL HEMATOLOGY - MEAN VALUES

INITIAL

<u>MALES</u>		ERYTHROCYTE COUNT ($10^6/\text{mm}^3$)	HEMOGLOBIN (gm%)	HEMATOCRIT (vol%)
DOSE LEVEL (PPM)	LEUKOCYTE COUNT ($10^3/\text{mm}^3$)			
0	9.52	6.120	14.37	41.88
100	11.20	6.817	15.70	46.00
300	9.12	6.790	15.77	46.38
1000	10.85	6.462	15.20	44.63
<u>FEMALES</u>				
0	10.32	7.200	16.77	49.38
100	10.42	6.645	16.10	46.88
300	9.75	6.945	16.42	48.25
1000	8.70	6.910	16.42	48.63

TABLE II-F-40 (Continued)

LITTON BIONETICS, INC.
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TABLE D (CONTINUED)

CLINICAL HEMATOLOGY - MEAN VALUES

WEEK 4

MALES

<u>DOSE LEVEL (PPM)</u>	<u>LEUKOCYTE COUNT ($10^3/\text{mm}^3$)</u>	<u>ERYTHROCYTE COUNT ($10^6/\text{mm}^3$)</u>	<u>HEMOGLOBIN (gm%)</u>	<u>HEMATOCRIT (vol%)</u>
0	10.45	6.930	15.42	43.75
100	10.40	7.390	15.75	45.25
300	10.62	7.240	16.00	44.13
1000	10.90	6.545	15.42	43.75

FEMALES

0	9.22	7.447	16.47	48.25
100	10.80	7.247	16.57	46.25
300	13.07	6.847	16.15	46.75
1000	12.60	6.972	16.47	46.63

TABLE 11-F-40 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE D (CONTINUED)

CLINICAL HEMATOLOGY - MEAN VALUES

WEEK 8

<u>MALES</u>		<u>ERYTHROCYTE COUNT</u> ($10^6/\text{mm}^3$)	<u>HEMOGLOBIN</u> (gm%)	<u>HEMATOCRIT</u> (vol%)
<u>DOSE LEVEL</u> (PPM)	<u>LEUKOCYTE COUNT</u> ($10^3/\text{mm}^3$)			
0	10.92	6.077	13.92	42.88
100	10.25	6.845	15.27	46.00
300	10.82	6.460	14.77	44.75
1000	10.22	6.687	14.42	44.25
<u>FEMALES</u>				
0	9.27	6.585	15.22	45.75
100	10.40	6.530	14.65	44.00
300	11.27	6.877	16.57	48.13
1000	10.82	6.440	14.92	44.63

TABLE II-F-40 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE D (CONTINUED)

CLINICAL HEMATOLOGY - MEAN VALUES

TERMINAL KILL

<u>MALES</u>					
<u>DOSE LEVEL</u> (PPM)	<u>LEUKOCYTE COUNT</u> ($10^3/\text{mm}^3$)	<u>ERYTHROCYTE COUNT</u> ($10^6/\text{mm}^3$)	<u>HEMOGLOBIN</u> (gm%)	<u>HEMATOCRIT</u> (vol%)	
0	9.62	6.765	15.25	42.75	
100	11.45	7.157	16.30	46.25	
300	9.37	6.967	16.00	45.63	
1000	9.47	6.857	15.05	43.63	
<u>FEMALES</u>					
0	10.10	7.175	15.92	45.25	
100	9.37	6.870	15.97	44.75	
300	12.52	7.365	16.05	45.13	
1000	10.45	6.922	16.20	46.75	

TABLE II-F-41
CLINICAL CHEMISTRY

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-09

TABLE E

INITIAL

MALES

DOSE LEVEL (PPM)	BILI- RUBIN, TOTAL (mg/dl)	BLOOD UREA NITROGEN (mg/dl)	ALBUMIN (g/dl)	CALCIUM (mg/dl)	CHOLE- STEROL (mg/dl)	GLUCOSE (mg/dl)	LACTIC DEHY- DROGENASE (mU/ml)	ALKALINE PHOS- PHATASE (mU/ml)	PHOS- PHORUS (mg/dl)	PROTEIN, TOTAL (g/dl)	SERUM GLUTAMIC- OXALACETIC TRANS- AMINASE (mU/ml)	SERUM GLUTAMIC- PYRUVIC TRANS- AMINASE (mU/ml)	URIC ACID (mg/dl)	SODIUM (g/dl)	POTASSIUM (mEq/l)	CHLORIDE (mEq/l)
0	0.07	13.5	3.80	11.12	148.5	96.5	282.0	75.8	4.90	6.25	34.8	37.5	0.40	147.3	5.10	107.5
100	0.05	15.8	3.77	11.12	162.3	84.3	144.8	88.0	5.07	6.32	31.5	37.5	0.40	146.3	4.70	108.0
300	0.07	11.5	4.20	11.07	174.0	90.5	166.8	69.0	4.47	6.55	30.5	39.0	0.45	145.0	4.82	107.5
1000	0.07	12.3	3.82	10.97	140.8	91.0	164.0	85.3	4.40	5.95	33.8	55.8	0.45	146.3	4.75	109.3

FEMALES

0	0.10	13.3	4.05	11.07	145.0	89.8	157.8	48.8	3.85	6.20	29.3	31.0	0.32	148.0	4.62	103.5
100	0.10	13.5	3.42	11.12	134.3	80.5	99.0	61.3	4.60	6.02	31.3	40.5	0.25	147.0	4.92	105.0
300	0.10	14.3	3.77	10.92	157.5	90.8	177.0	62.5	3.97	6.20	30.5	34.0	0.27	147.9	4.72	103.5
1000	0.07	13.5	4.02	10.95	146.8	83.5	145.5	69.3	5.20	5.92	31.8	43.3	0.35	147.3	4.72	103.8

TABLE II-F-41 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE E (CONTINUED)

WEEK 4

MALES

DOSE LEVEL (PPM)	BILI- RUBIN, TOTAL (mg/dl)	BLOOD UREA NITROGEN (mg/dl)	ALBUMIN (g/dl)	CALCIUM (mg/dl)	CHOLE- STEROL (mg/dl)	GLUCOSE (mg/dl)	LACTIC DEHY- DROGENASE (mU/ml)	ALKALINE PHOS- PHATASE (mU/ml)	PHOS- PHORUS (mg/dl)	PROTEIN, TOTAL (g/dl)	SERUM GLUTAMIC- OXALOACETIC TRANS- AMINASE (mU/ml)	SERUM GLUTAMIC- PYRUVIC TRANS- AMINASE (mU/ml)	URIC ACID (mg/dl)
0	0.10	15.5	3.10	10.82	135.3	86.0	290.3	58.0	4.40	6.20	54.3	33.5	0.22
100	0.07	16.5	3.02	10.92	164.5	83.5	257.3	64.8	4.20	6.22	63.8	33.8	0.17
300	0.10	15.3	2.90	10.77	149.0	84.5	206.5	61.5	4.30	6.20	51.3	35.3	0.20
1000	0.10	15.3	2.87	10.62	143.5	89.0	231.5	87.5	4.12	5.12	53.5	42.3	0.55

FEMALES

0	0.12	12.5	3.27	10.95	129.3	83.5	436.5	42.5	3.67	6.12	57.3	33.0	0.22
100	0.10	14.5	3.20	11.10	134.3	84.3	289.8	65.3	4.02	5.97	55.5	31.3	0.22
300	0.10	16.0	2.67	10.82	172.3	83.0	234.8	65.5	4.10	6.17	56.0	42.5	0.40
1000	0.10	16.8	3.02	11.00	163.3	77.5	346.8	69.0	4.12	6.05	58.8	40.0	0.37

TABLE II-F-41 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE E (CONTINUED)

WEEK 8

MALES

DOSE LEVEL (PPH)	BILI- RUBIN, TOTAL (mg/dl)	BLOOD UREA NITROGEN (mg/dl)	ALBUMIN (g/dl)	CALCIUM (mg/dl)	CHOLE- STEROL (mg/dl)	GLUCOSC (mg/dl)	LACTIC DEHY- DROGENASE (mU/ml)	ALKALINE PHOS- PHATASE (mU/ml)	PHOS- PHORUS (mg/dl)	PROTEIN, TOTAL (g/dl)	SERUM GLUTAMIC- OXALACETIC TRANS- AMINASE (mU/ml)	SERUM GLUTAMIC- PYRUVIC TRANS- AMINASE (mU/ml)	URIC ACID (mg/dl)
0	0.07	15.3	3.12	10.87	136.5	88.8	166.8	58.3	4.27	6.27	37.5	46.5	0.15
100	0.05	16.3	3.12	10.85	150.3	92.0	169.5	51.0	4.32	6.10	34.3	32.3	0.15
300	0.07	12.5	3.25	10.80	150.3	97.0	111.5	50.8	4.15	6.27	30.5	32.3	0.15
1000	0.10	12.0	3.07	10.45	114.8	87.3	147.5	57.5	4.12	5.07	36.5	41.5	0.02
<u>FEMALES</u>													
0	0.10	14.5	3.20	10.72	125.0	90.0	169.8	43.0	3.85	6.10	35.0	21.5	0.12
100	0.10	15.3	3.07	10.72	129.3	79.3	160.3	54.5	4.17	5.77	31.3	30.3	0.12
300	0.05	16.8	3.55	10.85	188.5	93.3	186.8	66.3	4.10	6.45	35.8	32.8	0.12
1000	0.12	16.8	3.15	10.60	166.8	82.3	187.3	61.8	4.20	5.90	35.3	29.3	0.15

TABLE II-F-41 (Continued)

ITION BIONETICS, INC.
PROJECT NO. 10734-09

TABLE E (CONTINUED)

TERMINAL KILL

MALES

DOSE LEVEL (PPH)	BILI- RUBIN, TOTAL (mg/dl)	BLOOD UREA NITROGEN (mg/dl)	ALBUMIN (g/dl)	CALCIUM (mg/dl)	CHOLE- STEROL (mg/dl)	GLUCOSE (mg/dl)	LACTIC DEHY- DROGENASE (mU/ml)	ALKALINE PHOS- PHATASE (mU/ml)	PHOS- PHORUS (mg/dl)	PROTEIN, TOTAL (g/dl)	SERUM GLUTAMIC- OXALOACETIC TRANS- AMINASE (mU/ml)	SERUM GLUTAMIC- PYRUVIC TRANS- AMINASE (mU/ml)	URIC ACID (mg/dl)
0	0.10	16.3	3.85	10.97	98.5	74.0	238.5	55.0	3.75	6.27	31.5	42.0	0.52
100	0.10	16.8	3.80	10.65	114.0	74.0	263.5	46.3	3.72	6.10	31.3	34.3	0.47
300	0.10	16.5	3.92	10.82	105.8	82.5	191.8	45.3	3.50	6.37	27.0	33.0	0.80
1000	0.10	15.5	3.75	10.67	92.8	87.5*	229.0	56.5	3.38	5.92	34.8	41.2	0.52

FEMALES

0	0.12	15.8	3.82	10.87	98.8	78.0	249.3	40.8	3.30	6.02	32.3	35.0	0.52
100	0.10	16.8	3.72	10.75	115.3	74.5	272.5	47.0	3.42	6.42	31.0	34.0	0.42
300	0.10	18.0	3.72	10.82	140.5	76.5	278.8	55.3	3.62	5.92	27.8	32.0	0.32
1000	0.12	17.5	3.80	10.85	120.0	74.5	256.5	54.0	3.60	5.82	29.8	31.0	0.32

* p<0.05 as compared to controls: Dunnett's t-test.

TABLE II-F-42

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE F

MEAN ORGAN WEIGHTS

<u>MALES</u>									
<u>DOSE LEVEL</u> <u>(PPM)</u>	<u>BODY</u> <u>WEIGHT</u>	<u>BRAIN</u>	<u>THYROID</u>	<u>HEART</u>	<u>LIVER</u>	<u>SPLEEN</u>	<u>KIDNEYS</u>	<u>ADRENALS</u>	<u>TESTES</u>
0	10875.0	78.5747	0.8747	86.8325	322.0172	77.7282	61.7987	1.1417	20.2657
100	11625.0	89.0372*	0.7730	88.0802	302.9150	95.4977	60.2855	1.0752	20.9540
300	9825.0	84.9087	0.6197	82.3457	272.0825	89.5575	53.7365	1.1215	19.1445
1000	10400.0	79.2218	0.6477	81.0655	285.9540	97.5097	55.9045	1.0907	18.6860
<u>FEMALES</u>									
<u>DOSE LEVEL</u> <u>(PPM)</u>	<u>BODY</u> <u>WEIGHT</u>	<u>BRAIN</u>	<u>THYROID</u>	<u>HEART</u>	<u>LIVER</u>	<u>SPLEEN</u>	<u>KIDNEYS</u>	<u>ADRENALS</u>	<u>OVARIES</u>
0	7550.0	75.4695	0.4940	67.6255	217.1627	80.7205	39.0597	0.8745	0.4472
100	8800.0	79.3445	0.6957	68.8920	255.7722	76.5630	40.9972	0.7627	1.4387
300	8850.0	78.2765	0.6425	64.5087	252.3592	91.7350	42.5392	0.8057	1.4852
1000	7925.0	72.4573	0.6137	65.9787	218.2760	91.6745	41.4102	0.7745	0.7730

* $p < 0.05$ as compared to controls: Dunnett's t-test.

TABLE II-F-43

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE G

MEAN ORGAN WEIGHT - BODY WEIGHT PERCENTAGES

MALES

<u>DOSE LEVEL (PPM)</u>	<u>BRAIN</u>	<u>THYROID</u>	<u>HEART</u>	<u>LIVER</u>	<u>SPLEEN</u>	<u>KIDNEYS</u>	<u>ADRENALS</u>	<u>TESTES</u>
0	0.7653	0.0095	0.8167	2.9828	0.7667	0.5799	0.0113	0.1949
100	0.7668	0.0067	0.7593	2.6139	0.8220	0.5196	0.0093	0.1813
300	0.8699	0.0062	0.8358	2.7619	0.9207	0.5484	0.0117	0.1945
1000	0.7642	0.0062	0.7803	2.7655	0.9378	0.5395	0.0107	0.1815

FEMALES

<u>DOSE LEVEL (PPM)</u>	<u>BRAIN</u>	<u>THYROID</u>	<u>HEART</u>	<u>LIVER</u>	<u>SPLEEN</u>	<u>KIDNEYS</u>	<u>ADRENALS</u>	<u>OVARIES</u>
0	1.0051	0.0065	0.9050	2.8928	1.0571	0.5148	0.0117	0.0061
100	0.9063	0.0079	0.7837	2.9104	0.8690	0.4668	0.0088	0.0164
300	0.9038	0.0074	0.7400	2.8789	1.0678	0.4887	0.0092	0.0155
1000	0.9190	0.0078	0.8359	2.7519	1.1658	0.5257	0.0098	0.0099

RESULTS (Continued)

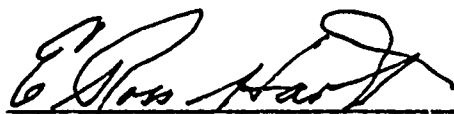
The reports of the consulting veterinary ophthalmologist have been included in the Appendix. The judgment of the ophthalmologist was that the compound produced no ocular changes in the dogs.

The pathological evaluation of tissues from dogs of the high dose and control groups has been included in the Appendix. This evaluation did not suggest any compound-related effect.

CONCLUSION

The test material, Dicyclopentadiene, was administered by incorporation into the diet at concentrations of 100, 300 and 1000 ppm to beagle dogs for 13 weeks. The animals were observed daily for general condition and behavior. Clinical pathological evaluations, including analysis of the clinical chemical constituents of serum, urine and hemograms, were performed at approximately monthly intervals. Tissues from the control and high dose dogs were histopathologically evaluated. Based on the results obtained using these criteria, it was concluded that treatment produced no significant toxicity with the possible exception of minor indications of intestinal distress expressed as vomiting and soft stools among dogs of the treated groups, especially the highest dose (1000 ppm).

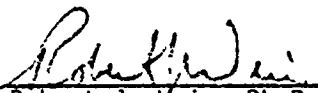
Submitted by:



E. Ross Hart, Ph.D.
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3/10/80
Date

Reviewed by:



Robert J. Weir, Ph.D.
Director
Department of Toxicology

3/11/80
Date

TABLE II-F-44

TABLE II-F-44 BY OBSERVATION REPORT

TABLE 1

PHYSICAL SIGNS

SUBJECT NUMBER - 1073409

DATE : 04/12/70

DATE	TIME	SEX	NUMBER	OBSERVATIONS : COMMENTS
001	0000	MALE	00417	SOFT STOOL
				STOOL LIQUID
	0000		00414	SOFT STOOL
				SOFT STOOL
		FEMALE	00421	SOFT STOOL
			00427	VOMITED-SEMI-SOLID
	1000	MALE	00416	NO FEED
				SOFT STOOL
			*****	NO FEED
				SOFT STOOL
			*****	NO FEED
			*****	WEIGHT LOSS-MARKED
			*****	FEED
				SOFT STOOL
			*****	WITH RED DISCHARGE
			00419	SOFT STOOL
			00420	SOFT STOOL
				SOFT STOOL
				VOMITED-SEMI-SOLID
				SOFT STOOL
			*****	WITH MUCUS
				VOMITED-SEMI-SOLID
		FEMALE	00423	SOFT STOOL
			00432	SOFT STOOL
				VOMITED-SEMI-SOLID
	002 1000	MALE	00405	LACERATION : LIMB-FLANK, RIGHT
			*****	FOOT PADS
			00415	VOMITED-LIQUID
	300	FEMALE	00433	ESTRUS
				ESTRUS
				ESTRUS
				ESTRUS
				ESTRUS
				ESTRUS
	1000	MALE	00413	MUCUS IN STOOL
				MUCUS IN STOOL
			00419	VOMITED-LIQUID
			00420	MUCUS IN STOOL
				MUCUS COATING STOOL
				VOMITED-LIQUID
				VOMITED-SEMI-SOLID
				MUCUS COATING STOOL

TABLE II-F-44 (Continued)

EXTRACT BY INSERVATION FORM

TABLE 1 (CONTINUED)

REF.	SEX	ANIMAL	OBSERVATIONS : QUALIFIER
1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100.
	FEMALE	00402	SOFT STOOL
003 CONTROL	MALE	00411	FECES-MUCOID
		00417	STOOL LIQUID
		*****	RED COLOR
			MUCUS IN STOOL
100 PPM	FEMALE	00404	SMALL ABCESS (<10 mm) : LING-FONE, RIGHT
	MALE	00405	VOMITED-LIQUID
		*****	YELLOW MUCUS
		00415	VOMITED-SEMI-SOLID
300 PPM	FEMALE	00424	FECES-MUCOID
		00427	ESTRUS
		00433	ESTRUS
			MUCUS IN STOOL
			ESTRUS
			MUCUS IN STOOL
1000 PPM	MALE	00416	VOMITED-LIQUID
			MUCUS IN STOOL
			FECES-MUCOID
		*****	RED MUCUS
			FECES-MUCOID
			FECES-MUCOID
			VOMITED-SEMI-SOLID
		*****	RED MUCUS
		00419	MUCUS IN STOOL
			FECES-MUCOID
		00420	MUCUS COATING STOOL
			MUCUS COATING STOOL
			MUCUS IN STOOL
			VOMITED-SEMI-SOLID
		*****	WITH MUCUS
			FECES-MUCOID
			VOMITED-SEMI-SOLID
			FECES-MUCOID
	FEMALE	00434	MUCUS IN STOOL
			SOFT STOOL
			FECES-MUCOID
		*****	STILL WITH RED
04 CONTROL	MALE	00417	STOOL LIQUID
		*****	RED
			STOOL LIQUID
		*****	MUCUS AND RED
			VOMITED-LIQUID
		*****	PHLEGM
100 PPM		00407	VOMITED-SEMI-SOLID
			FECES-MUCOID

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

DOSE	DOSE GROUP/SEX * NUMBER	ANIMAL	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
300 PPM	FEMALE	00415	FECES-MUCOID
		00431	FECES-MUCOID
	MALE	00404	FECES-MUCOID
			FECES-MUCOID
	FEMALE	00412	FECES-MUCOID
		00421	FECES-MUCOID
		00424	VOMITED-SEMISOLID
		00427	ESTRUS
		00433	FECES-MUCOID
			FECES-MUCOID
1000 PPM	MALE	00416	SOFT STOOL
			VOMITED-SEMISOLID
		SOFT STOOL	
		FECES-MUCOID	
	00419	FECES-MUCOID	
	00420	FECES-MUCOID	
	*****	YELLOW	
		FECES-MUCOID	
		SOFT STOOL	
		STOOL LIQUID	
	SOFT STOOL		
	SOFT STOOL		
	FECES-MUCOID		
FEMALE	00432	SOFT STOOL	
		SOFT STOOL	
	00436	EXCESS SHEDDING	
		EXCESS SHEDDING	
		ESTRUS	
005 CONTROL	MALE	00417	STOOL LIQUID
	*****	RED	
100 PPM	FEMALE	00434	ABSCCESS : LATERAL-RIGHT
		*****	FOREARM
	MALE	00407	SOFT STOOL
			VOMITED-SEMISOLID
		00415	FECES-MUCOID
		SOFT STOOL	
	FEMALE	00422	FECES-MUCOID
		00430	FECES-MUCOID
		00431	FECES-MUCOID
	300 PPM	MALE	00412
FEMALE		00427	VOMITED-LIQUID
	*****	PHLEGMA	
	00433	FECES-MUCOID	
		VOMITED-SEMISOLID	
1000 PPM	MALE	00416	FECES-MUCOID
		*****	RED AND YELLOW

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. ** DOSE GROUP/SEX * NUMBER	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
		FECES-MUCOID
	*****	YELLOW WITH RED
		SOFT STOOL
		FECES-MUCOID
	00420	SOFT STOOL
FEMALE	00423	FECES-MUCOID
	00432	SOFT STOOL
		SOFT STOOL
		FECES-MUCOID
		SOFT STOOL
		SOFT STOOL
	00436	ESTRUS
	00437	FECES-MUCOID
		VOMITED-SEMISOLID
005 CONTROL	MALE	00411 STOOL LIQUID
		FECES-MUCOID
	*****	YELLOW WITH RED
	00417	SOFT STOOL
	FEMALE	00434 SOFT STOOL
		00435 VOMITED-LIQUID
	*****	PHLEGM
100 PPM	MALE	00415 FECES-MUCOID
		SCAB : PENIS
	*****	SEVERAL
		SCAB : LIMB-HIND, RIGHT
	*****	SEVERAL
		SCAB : LIMB-HIND, LEFT
	*****	SEVERAL
	FEMALE	00422 SOFT STOOL
300 PPM		00424 SOFT STOOL
		00427 SOFT STOOL
		00433 VOMITED-SEMISOLID
	*****	MUCUS
1000 PPM	MALE	00413 VOMITED-LIQUID
	*****	PHLEGM
		00416 FECES-MUCOID
		SOFT STOOL
		FECES-MUCOID
		STOOL LIQUID
		FECES-MUCOID
	*****	YELLOW WITH RED
		SOFT STOOL
		VOMITED-LIQUID
	*****	PHLEGM
	00419	FECES-MUCOID
	00420	SOFT STOOL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. #	DOSE GROUP/SEX #	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
		FEMALE 00432	FECES-MUCOID VOMITED-SEMISOLID SOFT STOOL
007 CONTACT		MALE 00408	SOFT STOOL
		00417	SOFT STOOL
		*****	DARK RED
			VOMITED-LIQUID
		*****	YELLOW
			SOFT STOOL
		00418	FECES-MUCOID
		*****	VOMITED-LIQUID
			PHLEGM
		FEMALE 00425	SOFT STOOL
			SOFT STOOL
		00434	SOFT STOOL
		*****	ABSCESS : LIMB-FORE,RIGHT
			WITH SEVERAL SCABS
100 PPM		MALE 00405	FECES-MUCOID
			FECES-MUCOID
			FECES DISCOLORED
		*****	MUCUS IN STOOL
		00415	FECES-MUCOID
			LOCAL HAIR LOSS : LIMB-HIND,RIGHT
			LOCAL HAIR LOSS : LIMB-HIND,LEFT
			SMALL SCAB(1CM) : PENIS
		*****	SEVERAL
		FEMALE 00430	VOMITED-SEMISOLID
		00431	SOFT STOOL
			FECES-MUCOID
		*****	YELLOW MUCUS
300 PPM		MALE 00404	FECES-MUCOID
		FEMALE 00421	SOFT STOOL
		00424	SOFT STOOL
			FECES-MUCOID
		00433	FECES-MUCOID
1000 PPM		MALE 00416	STOOL LIQUID
			FECES-MUCOID
		*****	YELLOW WITH RED
			SOFT STOOL
			FECES-MUCOID
			STOOL LIQUID
			FECES-MUCOID
			SOFT STOOL
			FECES-MUCOID
		*****	WITH RED SPOTS
			SOFT STOOL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. **	DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
			FECES-MUCOID
		00420	SOFT STOOL
			SOFT STOOL
		*****	WITH MUCUS
	FEMALE	00432	SOFT STOOL
			STOOL LIQUID
			FECES-MUCOID
		00436	ESTRUS
			LOCAL HAIR LOSS : DORSAL
		*****	EXCESS SHEDDING
003	CONTROL	MALE	00408 URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00411	FECES-MUCOID
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00417	VOMITED-SEMISOLID
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00418	FECES-MUCOID
			URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
	FEMALE	00425	URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00426	URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00434	MEDIUM ABSCESS(1-5CM) : LIMB-FORE,RIGHT
		*****	SEVERAL ABSCESSES
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00435	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
100	PP4	MALE	00405 URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00406	URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00407	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00415	SMALL SCAB(<1CM) : PENIS
		*****	SEVERAL
			SMALL SCAB(<1CM) : LIMB-HIND,RIGHT
		*****	SEVERAL
			SMALL SCAB(<1CM) : LIMB-HIND,LEFT
		*****	SEVERAL
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
	FEMALE	00422	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00429	URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00430	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00431	FECES-MUCOID
			URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. ** DOSE GR/DJ/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
300 PPM	MALE 00404	FECES-MUCOID
		SOFT STOOL
		STOOL LIQUID
		URINE SUBMITTED FOR ANALYSIS
	00409	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		URINE SUBMITTED FOR ANALYSIS
	00412	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
	00414	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
	FEMALE 00421	FECES-MUCOID
		URINE SUBMITTED FOR ANALYSIS
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00424
		SOFT STOOL
		FECES-MUCOID
		SOFT STOOL
		SOFT STOOL
		FECES-MUCOID
		SOFT STOOL
		FECES-MUCOID
		SOFT STOOL
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
1000 PPM	MALE 00413	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00433
		FECES-MUCOID
		URINE SUBMITTED FOR ANALYSIS
	00416	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		URINE SUBMITTED FOR ANALYSIS
	00416	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		SOFT STOOL
		SOFT STOOL
		FECES-MUCOID
		VOMITED-LIQUID
		STOOL LIQUID
		FECES-MUCOID
		***** WITH RED STREAKS
		SOFT STOOL
		SOFT STOOL
		FECES-MUCOID
		URINE SUBMITTED FOR ANALYSIS
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00419
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
	00420	FECES-MUCOID
		SOFT STOOL
	FEMALE 00423	SOFT STOOL
		BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
	*****	FECES-MUCOID
		VOMITED-LIQUID
	*****	PHLEGM

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

ECK NO. **	DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER *** - COMMENTS *****
			URINE SUBMITTED FOR ANALYSIS
		00432	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM SOFT STOOL SOFT STOOL FECES-MUCOID SOFT STOOL SOFT STOOL FECES-MUCOID SOFT STOOL FECES-MUCOID BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00436	BLOOD SUBMITTED FOR CHEM AND HEMOGRAM EXCESS SHEDDING
		00437	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
000 CONTROL	MALE	00411	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
		00417	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM SOFT STOOL FECES-MUCOID FECES-MUCOID
	FEMALE	00434	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM FECES-MUCOID LOCAL HAIR LOSS : LIMB-FORE, RIGHT LESION : LIMB-FORE, RIGHT ***** SCM, RED
		00435	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
100 PPM	MALE	00406	SOFT STOOL SOFT STOOL
		00407	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM SOFT STOOL
		00415	URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM SOFT STOOL LOCAL HAIR LOSS : LIMB-HIND, RIGHT ***** INNER THIGH LOCAL HAIR LOSS : LIMB-HIND, LEFT ***** INNER THIGH ***** PENILE DISCHARGE ***** GREENISH ***** PAPILLOMA : PENIS ***** LEFT SIDE

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. **	DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
			LESION : PENIS LESION : LIMB-HIND, RIGHT ***** INNER THIGH LESION : LIMB-HIND, LEFT ***** INNER THIGH FEMALE 00422 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM SWELLING ***** MAMMARY GLANDS 00430 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM VOMITED-LIQUID ***** PHLEGM 300 PPM MALE 00404 SOFT STOOL FECES-MUCOID 00409 SOFT STOOL 00412 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM FECES-MUCOID VOMITED-LIQUID ***** PHLEGM FECES-MUCOID 00414 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM FEMALE 00421 SOFT STOOL 00424 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM 00427 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM FECES-MUCOID VOMITED-LIQUID ***** PHLEGM SWELLING ***** MAMMARY GLANDS 00432 FECES-MUCOID SOFT STOOL SWELLING ***** MAMMARY GLANDS 1000 PPM MALE 00416 FECES-MUCOID FECES-MUCOID ***** RED STREAKS 00419 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM LESION : LIMB-HIND, RIGHT ***** INNER THIGH, RED 00420 URINE SUBMITTED FOR ANALYSIS BLOOD SUBMITTED FOR CHEM AND HEMOGRAM

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. **	DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
			SOFT STOOL
	FEMALE	00432	STOOL LIQUID
			FECES-MUCOID
			URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
			SOFT STOOL
			SOFT STOOL
			STOOL LIQUID
			SOFT STOOL
			SOFT STOOL
		00436	URINE SUBMITTED FOR ANALYSIS
			BLOOD SUBMITTED FOR CHEM AND HEMOGRAM
			FECES-MUCOID
			EXCESS SHEDDING
		00437	SOFT STOOL
010 CONTROL	MALE	00403	FECES-MUCOID
			FECES-MUCOID
		00417	SOFT STOOL
			FECES-MUCOID
			SOFT STOOL
		00419	PENILE DISCHARGE
		*****	CLEANED W/PEROXIDE
	FEMALE	00426	FECES-MUCOID
			VOMITED-LIQUID
		*****	PHLEGM
			VOMITED-LIQUID
		*****	PHLEGM
		00434	LESION : LIMB-FORE, RIGHT
		*****	CLEANED W/PEROXIDE
			LESION : LIMB-FORE, RIGHT
		*****	CLEANED W/PEROXIDE
			LESION : LIMB-FORE, RIGHT
		*****	CLEANED W/PEROXIDE
			LESION : LIMB-FORE, RIGHT
		*****	CLEANED W/PEROXIDE
			LESION : LIMB-FORE, RIGHT
		*****	CLEANED W/PEROXIDE
			LESION
			LESION : LIMB-FORE, RIGHT
		*****	CLEANED W/PEROXIDE
		00435	VOMITED-LIQUID
		*****	PHLEGM
100 ppm	MALE	00407	SOFT STOOL
			SOFT STOOL
			VOMITED-LIQUID
		*****	PHLEGM

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	*****	OBSERVATIONS : QUALIFIER COMMENTS	*****
			*****	VOMITED-LIQUID	
			*****	PHLEGM	
			*****	SOFT STOOL	
			*****	VOMITED-LIQUID	
			*****	PHLEGM	
		00415	*****	PENILE DISCHARGE	
			*****	CLEANED W/PEROXIDE	
			*****	PENILE DISCHARGE	
			*****	CLEANED W/PEROXIDE	
			*****	PENILE DISCHARGE	
			*****	CLEANED W/PEROXIDE	
			*****	PENILE DISCHARGE	
			*****	CLEANED W/PEROXIDE	
			*****	SOFT STOOL	
			*****	PENILE DISCHARGE	
			*****	CLEANED W/PEROXIDE	
	FEMALE	00422	*****	SWELLING	
			*****	MAMMARY GLANDS	
			*****	SWELLING	
			*****	MAMMARY GLANDS	
			*****	SWELLING	
			*****	MAMMARY GLANDS	
			*****	SWELLING	
			*****	MAMMARY GLANDS	
			*****	SOFT STOOL	
			*****	SWELLING	
			*****	MAMMARY	
			*****	SWELLING	
			*****	MAMMARY GLANDS	
		00429	*****	SWELLING	
			*****	MAMMARY GLANDS	
			*****	SWELLING	
			*****	MAMMARY GLANDS	
		00430	*****	FECES-MUCOID	
			*****	VOMITED-LIQUID	
			*****	PHLEGM	
		00431	*****	SOFT STOOL	
			*****	FECES-MUCOID	
			*****	FECES-MUCOID	
			*****	FECES-MUCOID	
			*****	STOOL LIQUID	
			*****	FECES-MUCOID	
			*****	W/RED SPOTS	
			*****	FECES-MUCOID	
			*****	VOMITED-LIQUID	
			*****	PHLEGM	
	FEMALE	00424	*****	SOFT STOOL	

300 PPM

MALE

00404

FECES-MUCOID

STOOL LIQUID

FECES-MUCOID

FECES-MUCOID

FECES-MUCOID

FECES-MUCOID

FECES-MUCOID

FECES-MUCOID

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK	DOSE GROUP/SEX	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER
NO.	** DOSE GROUP/SEX *	*****	*****
		00427	SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
		00433	SWELLING
		*****	MAMMARY GLANDS
			STOOL LIQUID
			FECES-MUCOID
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
			VOMITED-LIQUID
		*****	PHLEGM
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
			SWELLING
		*****	MAMMARY GLANDS
1000 PPM	MALE	00416	SOFT STOOL
			FECES-MUCOID
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			FECES-MUCOID
		00420	SOFT STOOL
			VOMITED-LIQUID
		*****	PHLEGM
			SOFT STOOL
			VOMITED-LIQUID
		*****	PHLEGM
	FEMALE	00423	FECES-MUCOID
		*****	WITH RED STREAKS
			VOMITED-LIQUID
		*****	PHLEGM
		00432	SOFT STOOL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. **	DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
			SOFT STOOL
			FECES-MUCOID
			SOFT STOOL
			FECES-MUCOID
		*****	W/RED SPOTS
			SOFT STOOL
			FECES-MUCOID
			SOFT STOOL
			FECES-MUCOID
			SOFT STOOL
		00436	VOMITED-LIQUID
		*****	PHLEGM
		00437	VOMITED-LIQUID
		*****	PHLEGM
			SOFT STOOL
011	CONTROL	MALE	00408 STOOL LIQUID
			00411 SOFT STOOL
			00417 VOMITED-SEMISOLID
			SOFT STOOL
			STOOL LIQUID
			STOOL LIQUID
		00418	PENILE DISCHARGE
		*****	CLEANED W/PEROXIDE
			PENILE DISCHARGE
		*****	CLEANED W/PEROXIDE
			PENILE DISCHARGE
		*****	CLEANED W/PEROXIDE
			VOMITED-SEMISOLID
		00425	VOMITED-LIQUID
		*****	PHLEGM
			EYE ABNORMAL : EYE-RIGHT
		*****	RED
			EYE ABNORMAL : EYE-LEFT
		*****	RED
			EYE ABNORMAL : EYE-RIGHT
		*****	RED
			SOFT STOOL
			SOFT STOOL
			STOOL LIQUID
			SOFT STOOL
		00426	SOFT STOOL
		00434	LESION : LIMB-FORE,RIGHT
		*****	CLEANED W/PEROXIDE
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	DOSE GROUP/SEX	ANIMAL NUMBER	OBSERVATIONS : QUALIFIED COMMENTS	
		00420	SOFT STOOL SOFT STOOL STOOL LIQUID STOOL LIQUID STOOL LIQUID STOOL LIQUID STOOL LIQUID	
	FEMALE	00423	SOFT STOOL VOMITED-LIQUID	
		*****	PHLEGM	
		00432	SOFT STOOL STOOL LIQUID STOOL LIQUID FECES-MUCOID SOFT STOOL STOOL LIQUID STOOL LIQUID SOFT STOOL STOOL LIQUID APPEARS SKINNY	
		00436	SOFT STOOL SOFT STOOL SOFT STOOL SMALL MASS(KIDNEY) : LIMB-HIND, LEFT	
		*****	CLEANED W/ PEROXIDE	
		00437	SOFT STOOL VOMITED-SEMI-SOLID STOOL LIQUID FECES-MUCOID	
		*****	W/RED SPOTS	
			STOOL LIQUID SOFT STOOL SOFT STOOL SOFT STOOL	
013	CONTROL	MALE	00408	SOFT STOOL VOMITED-LIQUID
			*****	PHLEGM
		00411	FECES-MUCOID	
		00417	SOFT STOOL VOMITED-LIQUID	
		*****	PHLEGM	
			APPEARS SKINNY SOFT STOOL APPEARS SKINNY SOFT STOOL APPEARS SKINNY	

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	***** OBSERVATIONS : QUALIFIER COMMENTS	*****
			LESION : LIMB-FORE, RIGHT	
	100 PPM	MALE	***** CLEANED W/PEROXIDE	
		00405	SOFT STOOL	
			FECES-MUCOID	
		*****	COVERING STOOL	
		00406	VOMITED-LIQUID	
		*****	PHLEGM	
			SOFT STOOL	
		*****	STOOL SOFT W/MUCUS	
		00407	SOFT STOOL	
			STOOL LIQUID	
		00415	PENILE DISCHARGE	
		*****	CLEANED W/PEROXIDE	
			SOFT STOOL	
			FECES-MUCOID	
		*****	W/RED STREAKS	
			PENILE DISCHARGE	
		*****	CLEANED W/PEROXIDE	
			SOFT STOOL	
			PENILE DISCHARGE	
		*****	CLEANED W/PEROXIDE	
			PENILE DISCHARGE	
		*****	CLEANED W/PEROXIDE	
			PENILE DISCHARGE	
		*****	CLEANED W/PEROXIDE	
			PAPILLOMA : PENIS	
		*****	LEFT SIDE	
			LESION : LIMBS-HIND	
		*****	BOTH LEFT/RT THIGHS	
			SMALL SCAB(1CM) : PENIS	
		*****	SEVERAL	
	FEMALE	00422	SOFT STOOL	
			FECES-MUCOID	
			SOFT STOOL	
			SWELLING : VENTRAL-MID	
		*****	MAMMARY GLANDS	
		00430	VOMITED-SEMI-SOLID	
		*****	ESTRUS	
			SOFT STOOL	
			STOOL LIQUID	
			SOFT STOOL	
	300 PPM	MALE	00431	FECES-MUCOID
		00404	FECES-MUCOID	
			SOFT STOOL	
			SOFT STOOL	
			FECES-MUCOID	
		00409	VOMITED-LIQUID	
		*****	PHLEGM	

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. **	DOSE	GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
				PENILE DISCHARGE
			*****	CLEANED W/PEROXIDE
			*****	PENILE DISCHARGE
			*****	CLEANED W/PEROXIDE
			00412	VOMITED-LIQUID
			*****	PHLEGA
				STOOL LIQUID
		FEMALE	00421	SOFT STOOL
				FECES-MUCOID
			*****	LIQUID MUCUS IN STOOL
			00424	VOMITED-LIQUID
			*****	PHLEGA
				FECES-MUCOID
			00427	STOOL LIQUID
			*****	STOOL W/MUCUS
			00433	STOOL LIQUID
				FECES-MUCOID
				SWELLING
			*****	MAMMARY GLANDS
				SWELLING : VENTRAL-MID
			*****	MAMMARY GLANDS
				SWELLING : VENTRAL-MID
			*****	MAMMARY GLANDS
				SWELLING : VENTRAL-MID
			*****	MAMMARY GLANDS
				SWELLING : VENTRAL-MID
			*****	MAMMARY GLANDS
				SWELLING : VENTRAL-MID
			*****	MAMMARY GLANDS
				SWELLING : VENTRAL-MID
			*****	MAMMARY GLANDS
1000 PPM		MALE	00413	SOFT STOOL
				PENILE DISCHARGE
			*****	CLEANED W/PEROXIDE
			00416	STOOL LIQUID
				FECES-MUCOID
				SOFT STOOL
				SOFT STOOL
				PENILE DISCHARGE
			*****	CLEANED W/PEROXIDE
			00419	FECES-MUCOID
				SOFT STOOL
				LOCAL HAIR LOSS : LIMB-HIND, RIGHT
			*****	INNER THIGH
				PAPILLOMA : PENIS
			*****	LEFT SIDE
				LESION : LIMB-HIND, RIGHT
			*****	SMALL/SEVERAL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
		00420	FECES-MUCOID SOFT STOOL FECES-MUCOID ***** IN SOFT STOOL VOMITED-LIQUID ***** PHLEGM SOFT STOOL SOFT STOOL ***** COVERED W/MUCUS STOOL LIQUID ***** W/MUCUS SOFT STOOL
	FEMALE	00423	SOFT STOOL
		00432	SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL STOOL LIQUID SOFT STOOL
		00437	SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL
012	CONTROL	MALE	00408 SOFT STOOL VOMITED-LIQUID ***** PHLEGM 00411 SOFT STOOL 00417 STOOL LIQUID STOOL LIQUID STOOL LIQUID SOFT STOOL SOFT STOOL FECES-MUCOID SOFT STOOL APPEARS SKINNY
	FEMALE	00413	SOFT STOOL
		00425	STOOL LIQUID
		00426	VOMITED-LIQUID ***** PHLEGM
		00434	SOFT STOOL LOCAL HAIR LOSS : LIMB-FORE, RIGHT SOFT STOOL LESION : LIMB-FORE, RIGHT

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. **	DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
100	2PM	MALE	00405 VOMITED-LIQUID ***** PHLEGM SOFT STOOL SOFT STOOL PAPILLOMA : NOSE: ABOVE
			00406 STOOL LIQUID VOMITED-SEMISOLID VOMITED-SEMISOLID SOFT STOOL
			00407 SWELLING : LIMB-HIND, LEFT ***** ON FOOT LESION : LIMB-HIND, LEFT ***** CLEANED W/PEROXIDE
			00415 SOFT STOOL FECES-MUCRID PENILE DISCHARGE ***** CLEANED W/PEROXIDE STOOL LIQUID STOOL LIQUID LOCAL HAIR LOSS : PENIS LOCAL HAIR LOSS : LIMB-HIND, RIGHT ***** VENTRAL SIDE LOCAL HAIR LOSS : LIMB-HIND, LEFT ***** VENTRAL SIDE PENILE DISCHARGE SOFT STOOL PAPILLOMA : PENIS ***** LEFT AND RIGHT SIDES
		FEMALE	00422 SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SOFT STOOL SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SWELLING : VENTRAL-MID ***** MAMMARY GLANDS 00429 SOFT STOOL SOFT STOOL 00430 STOOL LIQUID STOOL LIQUID

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

DOSE	DOSE GROUP/SEX	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****	
300 PPM	MALE	00431	FECES-MUCOID SOFT STOOL	
		00404	SOFT STOOL SOFT STOOL VOMITED-SEMISOLID STOOL LIQUID FECES-MUCOID	
		00409	PENILE DISCHARGE ***** CLEANED W/PEROXIDE SOFT STOOL SOFT STOOL PENILE DISCHARGE SOFT STOOL	
		00412	SOFT STOOL STOOL LIQUID	
		FEMALE	00421	SOFT STOOL SOFT STOOL SOFT STOOL SCARPED TISSUE : EAR-LEFT:INSIDE ***** ON FLAP-KED AREA-1.5
			00424	SOFT STOOL SOFT STOOL SOFT STOOL FECES-MUCOID
			00427	SOFT STOOL VOMITED-SEMISOLID
			00433	SWELLING : VENTRAL-MID ***** MAMMARY GLANDS SWELLING : VENTRAL-MID ***** MAMMARY GLANDS
			00413	SOFT STOOL SOFT STOOL VOMITED-LIQUID ***** PHLEGM
	MALE	00413	SOFT STOOL STOOL LIQUID	
		00416	SOFT STOOL FECES-MUCOID SOFT STOOL STOOL LIQUID STOOL LIQUID SOFT STOOL	
		00419	STOOL LIQUID FECES-MUCOID FECES-MUCOID LOCAL HAIR LOSS : LIMB-HIND,RIGHT ***** INNER THIGH	

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
			SOFT STOOL
			APPEARS SKINNY
			SOFT STOOL
			APPEARS SKINNY
			SOFT STOOL
			APPEARS SKINNY
			SOFT STOOL
	FEMALE	00425	SOFT STOOL
			SOFT STOOL
		00426	SOFT STOOL
			SOFT STOOL
		00434	SOFT STOOL
			SOFT STOOL
100 PPM	MALE	00405	SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
		00406	SOFT STOOL
			FECES-MUCOID
		00407	LESION : LIMB-HIND, LEFT
		*****	CLEANED W/PEROXIDE
			SOFT STOOL
			SOFT STOOL
			VOMITED-LIQUID
		*****	PHLEGM
		00415	STOOL LIQUID
			FECES-MUCOID
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
	FEMALE	00422	SWELLING : VENTRAL-MID
		*****	MAMMARY GLANDS
			SWELLING : VENTRAL-MID
			SOFT STOOL
			SWELLING : VENTRAL-MID
		*****	MAMMARY GLANDS
			SOFT STOOL
			SWELLING : VENTRAL-MID
			SWELLING : VENTRAL-MID
		*****	MAMMARY GLANDS
			SWELLING : VENTRAL-MID
		*****	MAMMARY GLANDS
			SWELLING : VENTRAL-MID
		*****	MAMMARY GLANDS
		00429	SOFT STOOL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
			SOFT STOOL
			SOFT STOOL
		00430	STOOL LIQUID
			VOMITED-SEMISOLID
			SOFT STOOL
			STOOL LIQUID
			STOOL LIQUID
300 PPM	MALE	00404	SOFT STOOL
		00409	STOOL LIQUID
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
		00412	STOOL LIQUID
			SOFT STOOL
		00414	SOFT STOOL
			VOMITED-SEMISOLID
			SOFT STOOL
	FEMALE	00421	SOFT STOOL
			SOFT STOOL
			SOFT STOOL
		00424	SOFT STOOL
			SOFT STOOL
			SOFT STOOL
		00433	STOOL LIQUID
			SOFT STOOL
			FECES-MUCOID
			SOFT STOOL
			SOFT STOOL
1000 PPM	MALE	00416	SOFT STOOL
			SOFT STOOL
		00420	SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			VOMITED-LIQUID
		*****	PHLEGM
			SOFT STOOL
	FEMALE	00423	SOFT STOOL
		00432	SOFT STOOL
			APPEARS SKINNY
			STOOL LIQUID
			APPEARS SKINNY
			STOOL LIQUID
			FECES-MUCOID

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. ** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS *****
		APPEARS SKINNY
		STOOL LIQUID
		FECES-MUCOID
		APPEARS SKINNY
		STOOL LIQUID
		APPEARS SKINNY
		STOOL LIQUID
		APPEARS SKINNY
		STOOL LIQUID
		SKINNY
	00436	SOFT STOOL
	00437	SOFT STOOL
		SOFT STOOL
		STOOL LIQUID
		SOFT STOOL
		FECES-MUCOID
	*****	LIQUID MUCUS
		SOFT STOOL
014 CONTROL	MALE	00403
		SOFT STOOL
		VOMITED-LIQUID
	*****	PHLEGM
		VOMITED-LIQUID
	*****	PHLEGM
		TERMINAL KILL
	00411	TERMINAL KILL
	00417	SOFT STOOL
		SKINNY
		SOFT STOOL
		SKINNY
		SOFT STOOL
		VOMITED-LIQUID
	*****	PHLEGM
		SOFT STOOL
		SKINNY APPEARANCE
		STOOL LIQUID
		SKINNY
		TERMINAL KILL
	00413	TERMINAL KILL
FEMALE	00425	SOFT STOOL
		SOFT STOOL
		SOFT STOOL
		SOFT STOOL
		SOFT STOOL
		TERMINAL KILL
		TERMINAL KILL
		SOFT STOOL

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK	DOSE	GROUP/SEX	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER COMMENTS
100 PPM	MALE	00426	SOFT STOOL TERMINAL KILL	
		00434	SOFT STOOL	
		00405	SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL SOFT STOOL TERMINAL KILL TERMINAL KILL	
		00406	SOFT STOOL SOFT STOOL STOOL LIQUID TERMINAL KILL	
		00407	SOFT STOOL FECES-MUCOID	
	FEMALE	00415	SOFT STOOL	
		00422	SWELLING : VENTRAL-MID MAMMARY GLANDS SWELLING : VENTRAL-MID MAMMARY GLANDS SWELLING : VENTRAL-MID MAMMARY GLANDS SOFT STOOL SWELLING : VENTRAL-MID MAMMARY GLANDS SOFT STOOL SWELLING : VENTRAL-MID MAMMARY GLANDS TERMINAL KILL SOFT STOOL SWELLING : VENTRAL-MID MAMMARY GLANDS 00429 VOMITED-SEMISOLID SOFT STOOL SOFT STOOL TERMINAL KILL SOFT STOOL	
		00430	SOFT STOOL TERMINAL KILL	
		00431	SOFT STOOL TERMINAL KILL	
300 PPM	MALE	00404	TERMINAL KILL STOOL LIQUID	
		00409	SOFT STOOL SOFT STOOL SOFT STOOL	

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO.	** DOSE GROUP/SEX *	ANIMAL NUMBER	OBSERVATIONS : QUALIFIED ***** COMMENTS *****
			TERMINAL KILL
		00412	SOFT STOOL
			TERMINAL KILL
		00414	TERMINAL KILL
	FEMALE	00421	STOOL LIQUID
			ESTRUS
			TERMINAL KILL
		00424	SOFT STOOL
			SOFT STOOL
			STOOL LIQUID
			TERMINAL KILL
		00433	SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			FECESES-MUCOID
1000 PPM	MALE	00413	VOMITED-LIQUID
		*****	PHLEGM
			VOMITED-LIQUID
		*****	PHLEGM
			TERMINAL KILL
		00416	SOFT STOOL
			STOOL LIQUID
			SOFT STOOL
			TERMINAL KILL
		00419	FECESES-MUCOID
		00420	SOFT STOOL
			SOFT STOOL
			SOFT STOOL
			VOMITED-SEMISOLID
			SOFT STOOL
			SOFT STOOL
	FEMALE	00423	SOFT STOOL
			VOMITED-LIQUID
		*****	PHLEGM
			STOOL LIQUID
			SOFT STOOL
			TERMINAL KILL
		00432	STOOL LIQUID
			SKINNY
			STOOL LIQUID
			SKINNY
			STOOL LIQUID
			SKINNY APPEARANCE
			STOOL LIQUID
			SKINNY APPEARANCE
			STOOL LIQUID
			SKINNY APPEARANCE

TABLE II-F-44 (Continued)

TOXICOLOGY OBSERVATION REPORT

TABLE 1 (CONTINUED)

WEEK NO. ** DOSE GROUP/SEX -	ANIMAL NUMBER	OBSERVATIONS : QUALIFIER ***** COMMENTS
		TERMINAL KILL
		STOOL LIQUID
		SKINNY APPEARANCE
	00436	VOMITED-SEMISOLID
		RED SPOTS IN STOOL
		TERMINAL KILL
	00437	STOOL LIQUID
		STOOL LIQUID
		SOFT STOOL
		SOFT STOOL
		TERMINAL KILL
015 CONTROL	FEMALE	00434 TERMINAL KILL
		00435 TERMINAL KILL
100 PPM	MALE	00407 TERMINAL KILL
		00415 TERMINAL KILL
300 PPM	FEMALE	00427 TERMINAL KILL
		00433 TERMINAL KILL
1000 PPM	MALE	00419 TERMINAL KILL
		00420 TERMINAL KILL

TABLE II-F-45

PROJECT NO. 1073409
 COMPUTER GROUP NO: 301A
 LITTON BIONETICS, INC.
 BODY WEIGHT DATA
 GROUP 1 MALES

DOSE: CONTROL

ANML NO.	TABLE 2 DATES OF TESTING (1978)															TERMINATION
	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9		
408	13.6	13.6	13.5	14.0	14.1	13.8	14.3	14.4	14.5	15.3	15.4	15.5	16.0	15.5	15.8	
411	9.8	9.7	9.5	9.8	10.4	9.8	9.8	9.9	9.8	9.9	10.0	10.1	10.5	9.8	9.8	
417	10.0	9.8	9.7	9.6	10.2	9.4	9.4	9.5	9.5	9.3	9.6	9.2	10.0	9.3	9.2	
418	9.6	9.8	9.7	9.8	9.5	9.4	9.5	9.4	8.9	9.9	9.8	9.8	9.8	9.5	8.7	
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
MEAN	10.8	10.7	10.6	10.8	11.0	10.6	10.8	10.8	10.7	11.1	11.2	11.1	11.6	11.0	10.9	
S.D.	1.9	1.9	1.9	2.1	2.1	2.1	2.4	2.4	2.6	2.8	2.8	2.9	3.0	3.0	3.3	
S.E.	1.0	1.0	1.0	1.1	1.0	1.1	1.2	1.2	1.3	1.4	1.4	1.5	1.5	1.5	1.7	

TABLE II-F-45 (Continued)

PROJECT NO. 10/3409 COMPUTER GROUP NO: 301B LITTON BIONETICS, INC. BODY WEIGHT DATA GROUP 1 FEMALES DOSE: CONTROL															
TABLE 2 (CONTINUED) ANML NO. DATES OF TESTING (1978)															
	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	TERMINATION
425	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
426	8.6	8.4	8.1	8.5	8.2	7.8	8.3	8.3	8.2	8.3	8.6	8.6	8.6	8.3	8.3
434	8.8	7.9	7.9	8.1	8.1	7.7	7.6	7.6	7.5	7.9	8.1	7.7	8.1	7.7	7.6
435	8.1	7.8	7.7	7.9	8.3	8.0	7.9	7.9	7.9	8.1	8.5	8.5	8.4	7.9	7.9
	7.0	6.9	6.7	6.9	6.9	6.4	6.6	6.7	6.9	6.9	6.9	6.9	6.8	6.4	6.4
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MEAN	8.1	7.7	7.6	7.8	7.9	7.5	7.6	7.6	7.6	7.8	8.0	7.9	8.0	7.6	7.5
S.D.	0.8	0.6	0.6	0.7	0.7	0.7	0.7	0.7	0.6	0.6	0.8	0.8	0.8	0.8	0.8
S.E.	0.4	0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4	0.4	0.4

TABLE II-F-45 (Continued)

PROJECT NO. 1073409
 COMPUTER GROUP NO: 301C
 LITTON BIONETICS, INC.
 BODY WEIGHT DATA
 GROUP 2 MALES DOSE: 100 PPM

TABLE 2 (CONTINUED)		DATES OF TESTING (1978)												TERMINATION	
ANWL NO.	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	14
405	12.4	11.7	11.5	11.9	12.7	11.0	11.5	11.9	12.0	12.3	12.1	12.7	12.6	11.8	12.0
406	12.8	12.7	12.6	13.0	12.7	12.7	13.1	13.4	12.9	11.6	13.4	13.6	14.1	13.6	12.4
407	11.8	11.5	11.7	11.3	11.6	11.7	11.5	11.6	12.1	13.4	11.6	11.8	11.1	11.0	10.9
415	11.4	11.4	11.4	11.7	11.5	11.7	11.8	11.7	11.7	11.8	11.9	11.9	12.0	11.3	11.2
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MEAN	12.1	11.8	11.8	12.0	12.2	11.8	12.0	12.2	12.2	12.3	12.3	12.5	12.4	11.9	11.6
S.D.	0.6	0.6	0.5	0.7	0.6	0.7	0.8	0.8	0.5	0.8	0.8	0.8	1.3	1.2	0.7
S.E.	0.3	0.3	0.3	0.4	0.3	0.3	0.4	0.4	0.3	0.4	0.4	0.4	0.6	0.6	0.3

TABLE II-F-45 (Continued)

PROJECT NO. 1073409															
COMPUTER GROUP NO: 301D															
LITTON BIONETICS, INC.															
BODY WEIGHT DATA															
GROUP 2 FEMALES															
DOSE: 100 PPM															
TABLE 2 (CONTINUED)															
ANAL DATES OF TESTING (1978)															
NO.	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	TERMINATION
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
422	9.0	8.7	8.5	8.7	8.7	8.5	8.6	9.0	8.8	9.1	8.8	8.9	9.1	8.6	8.7
429	9.0	9.0	8.8	8.9	9.1	9.1	9.3	8.9	9.0	9.1	9.3	9.2	9.4	9.6	9.6
430	9.9	9.7	9.9	9.9	10.0	9.4	9.9	10.1	10.0	10.2	10.1	9.8	10.3	9.7	9.1
431	8.0	7.9	7.8	7.9	8.4	7.6	7.9	8.2	8.1	8.6	8.6	8.5	8.5	8.3	7.8
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MEAN	9.0	8.8	8.8	8.8	9.0	8.6	8.9	9.0	9.0	9.3	9.2	9.1	9.3	9.1	8.8
S.D.	0.8	0.7	0.9	0.8	0.7	0.8	0.9	0.8	0.8	0.7	0.7	0.5	0.8	0.7	0.8
S.E.	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.3	0.3	0.3	0.4	0.4	0.4

TABLE II-F-45 (Continued)

PROJECT NO. 1073409
 COMPUTER GROUP NO: 301E
 LITTON BIONETICS, INC.
 BODY WEIGHT DATA
 GROUP 3 MALES DOSE: 300 PPM

TABLE 2 (CONTINUED)		TESTING (1978)												TERMINATION	
NO.	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	
404	10.6	10.4	10.2	10.3	10.9	10.0	10.7	10.9	10.4	10.5	10.9	11	12	13	14
409	9.4	9.5	9.1	9.4	9.7	9.7	9.7	9.5	9.4	9.8	9.8	9.8	11.1	10.4	10.8
412	9.2	9.0	9.0	9.2	9.1	8.9	9.0	9.4	9.5	9.5	9.5	9.2	10.0	9.2	9.4
414	10.7	10.5	10.5	10.9	10.6	10.5	10.7	11.1	11.2	11.3	11.4	11.5	11.5	10.8	10.4
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MEAN	10.0	9.8	9.7	9.9	10.1	9.8	10.0	10.2	10.1	10.3	10.4	10.3	10.4	9.9	9.8
S.D.	0.8	0.7	0.8	0.8	0.8	0.7	0.8	0.9	0.8	0.8	0.9	1.0	1.2	0.9	1.0
S.E.	0.4	0.4	0.4	0.4	0.4	0.3	0.4	0.4	0.4	0.4	0.4	0.5	0.6	0.4	0.5

TABLE II-F-45 (Continued)

PROJECT NO. 1073409
 COMPUTER GROUP NO: 301F
 LITTON BIONETICS, INC.
 BODY WEIGHT DATA
 GROUP 3 FEMALES
 TABLE 2 (CONTINUED)
 DOSE: 300 PPM

ANHL NO.	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	TERMINATION
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
421	10.6	10.5	10.1	10.4	10.5	10.4	10.6	10.8	10.3	11.0	10.8	10.9	11.1	10.5	10.9
424	8.5	8.1	8.3	8.6	9.0	8.5	8.3	8.4	8.9	8.7	8.7	9.1	8.6	8.4	7.8
427	7.3	7.3	7.2	7.5	7.6	7.6	7.5	7.6	7.5	7.5	7.7	7.7	7.6	7.4	7.3
433	8.9	9.3	9.3	9.2	10.1	9.8	9.6	9.8	9.5	10.3	10.3	10.4	9.9	9.4	9.4
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MEAN	8.8	8.8	8.7	8.9	9.3	9.1	9.0	9.1	9.0	9.4	9.4	9.5	9.3	8.9	8.8
S.D.	1.4	1.4	1.3	1.2	1.3	1.3	1.4	1.4	1.2	1.6	1.4	1.4	1.5	1.3	1.6
S.E.	0.7	0.7	0.6	0.6	0.6	0.6	0.7	0.7	0.6	0.8	0.7	0.7	0.8	0.7	0.8

TABLE II-F-45 (Continued)

PROJECT NO. 1073409
 COMPUTER GROUP NO: 301G
 LITTON BIONETICS, INC.
 BODY WEIGHT DATA
 GROUP 4 MALES
 DOSES: 1000 PPM

TABLE 2 (CONTINUED)		ANNL DATES OF TESTING (1978)												TERMINATION	
NU.	5/10	5/17	5/24	5/31	6/7	6/14	6/21	6/28	7/5	7/12	7/19	7/26	8/2	8/9	
413	10.9	10.6	10.6	9.9	10.5	10.2	10.5	10.5	10.5	10.8	10.9	11.1	12	13	14
416	10.0	9.9	10.2	9.9	9.8	9.7	9.7	10.0	9.6	10.3	10.2	10.2	10.9	10.3	10.7
419	11.0	10.8	10.9	11.1	11.1	10.7	11.0	11.3	10.7	10.5	10.2	10.3	10.6	9.8	9.4
420	11.4	10.9	11.4	11.5	12.3	11.7	11.5	11.1	11.2	10.9	11.2	11.5	11.1	10.6	10.6
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MEAN	10.8	10.5	10.8	10.6	10.9	10.6	10.7	10.7	10.5	10.6	10.6	10.7	10.9	10.4	10.4
S.D.	0.6	0.5	0.5	0.8	1.1	0.9	0.8	0.6	0.7	0.3	0.5	0.6	0.3	0.5	0.7
S.E.	0.3	0.2	0.3	0.4	0.5	0.4	0.4	0.3	0.3	0.1	0.3	0.3	0.1	0.3	0.3

TABLE II-F-45 (Continued)

PROJECT NR. 10/3409
COMPUTER GROUP NO: 301H
LITTON ELECTRONICS, INC.
JULY WEIGHT DATA
GROUP 4 FEMALES DOSE: 1000 PPM

TABLE 2 (CONTINUED)																
ANML NO.	TESTING (1978)										TERMINATION					
	5/10	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9		
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
423	7.8	7.6	7.5	7.7	10.2	7.4	7.7	7.9	7.5	8.0	7.8	7.9	8.0	7.3	7.6	
432	8.0	7.9	7.8	7.8	7.8	7.6	7.6	7.9	7.8	7.9	7.8	7.9	7.9	7.4	7.4	
436	8.4	8.0	7.9	7.9	8.2	8.2	8.4	8.5	8.8	8.3	8.2	8.0	8.1	8.0	7.9	
437	9.0	8.8	8.9	8.9	9.3	9.2	9.0	9.2	9.0	9.2	9.4	9.6	9.8	8.9	8.8	
SAMPLE	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	
MEAN	8.3	8.1	8.0	8.1	8.9	8.1	8.2	8.4	8.3	8.3	8.3	8.3	8.4	7.9	7.9	
S.D.	0.5	0.5	0.6	0.6	1.1	0.8	0.7	0.6	0.7	0.6	0.8	0.8	0.9	0.7	0.6	
S.E.	0.3	0.3	0.3	0.3	0.5	0.4	0.3	0.3	0.4	0.3	0.4	0.4	0.5	0.4	0.3	

TABLE II-F-46

PROJECT NO. 1073409
 COMPUTER GROUP NO: 301A
 LITTON BIONETICS, INC.
 DAILY FOOD INTAKE IN GRAMS
 GROUP 1 MALES DOSE: CONTROL

TABLE 3														
ANML NO.	DATES OF TESTING (1978)													
	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	8/16
408	382.3	337.3	433.7	-	-	-	-	479.7	447.6	395.1	-	388.1	368.3	411.1
411	-	-	-	-	-	-	-	-	-	-	-	-	261.1	302.2
417	310.8	290.6	289.5	289.0	371.4	-	300.4	333.5	240.1	314.9	251.3	360.4	285.9	350.4
418	346.1	345.6	376.3	266.9	316.8	297.2	294.4	288.7	371.2	325.2	340.9	340.8	345.3	324.2
SAMPLE	3	3	3	2	2	1	2	3	3	3	2	3	4	4
MEAN	346.4	324.5	366.5	278.0	344.1	297.2	297.4	367.3	353.0	345.1	296.1	363.1	315.2	347.0
S.D.	35.8	29.6	72.6	15.6	30.6	0.0	4.2	99.9	104.9	43.7	63.4	23.8	50.0	47.0
S.E.	20.7	17.1	41.9	11.0	27.3	0.0	3.0	57.7	60.6	25.2	44.8	13.7	25.0	23.5

TABLE II-F-46 (Continued)

PROJECT NO. 1073409 COMPUTER GROUP NO: 301B LITTON HIGNETICS, INC. DAILY FOOD INTAKE IN GRAMS GROUP 1 FEMALES DOSE: CONTROL TABLE3 (CONTINUED)														
ANML NO.	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	8/16
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
425	348.2	-	-	-	309.4	-	-	263.5	-	266.8	-	-	297.3	263.4
426	226.6	247.1	283.7	264.6	-	-	-	-	300.1	271.8	-	259.0	288.1	274.9
434	262.0	271.6	320.2	283.7	424.2	-	304.9	326.5	328.5	324.1	350.2	299.1	-	368.8
435	214.4	208.0	199.0	191.9	302.4	-	-	215.5	216.0	-	-	197.2	220.5	265.7
SAMPLE	4	3	3	3	3	0.0	1	3	3	3	1	3	3	4
MEAN	262.8	242.2	261.7	246.7	345.3	0.0	304.9	268.5	281.5	287.6	350.2	251.8	268.6	293.2
S.D.	60.4	32.1	62.2	48.5	68.4	0.0	0.0	55.7	58.5	31.7	0.0	51.4	42.0	50.6
S.E.	30.2	18.5	31.9	24.0	39.5	0.0	0.0	32.1	31.8	18.3	0.0	29.6	24.2	25.3

^aAll spilled food.

TABLE II-F-46 (Continued)

PROJECT NO. 1073409															
COMPUTER GROUP NO: 101C															
LITTON BIOINFORMATICS, INC.															
DAILY FOOD INTAKE IN GRAMS															
GROUP 2 MALES															
TABLE 3 (CONTINUED)															
DOSE: 100 PPM															
ANAL DATES OF TESTING (1978)															
NO.	5/17	5/24	5/31	6/7	6/14	6/21	6/28	7/5	7/12	7/19	7/26	8/2	8/9	8/16	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
405	411.4	387.9	-	-	-	-	-	-	383.8	326.0	357.8	365.7	354.9	317.0	
406	309.1	-	-	-	414.4	312.5	361.5	265.3	338.6	314.9	300.6	304.8	340.3	450.7	
407	304.4	355.6	302.3	361.3	313.2	386.0	348.5	349.8	-	-	-	-	358.6	252.5	
415	327.1	317.9	337.6	-	-	-	278.8	265.0	306.8	308.2	271.9	258.5	225.5	311.0	
SAMPLE	4	3	2	1	2	2	3	3	3	3	3	3	4	4	
MEAN	338.0	353.8	319.9	361.3	363.0	349.2	329.6	293.4	343.1	316.4	310.1	309.7	319.8	332.8	
S.D.	49.9	35.0	25.0	0.0	71.6	52.0	44.5	48.9	38.7	9.0	43.7	53.7	63.4	83.8	
S.E.	24.9	20.2	17.6	0.0	50.6	36.8	25.1	28.2	22.3	5.2	25.2	31.0	31.7	41.9	

TABLE II-F-46 (Continued)

PROJECT NO. 10/5609														
COMPUTER GROUP NO: 301D														
LITTON BIOMEDICS, INC.														
DAILY FOOD INTAKE IN GRAMS														
GROUP 2 FEMALES DOSE: 100 PPM														
TABLE 3 (CONTINUED)														
ANML DATES OF TESTING (1976)														
NO.	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	8/16
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
422	213.4	201.9	238.0	207.3	201.1	215.6	246.4	213.9	198.4	161.5	218.4	203.4	180.5	210.6
429	262.9	-	285.6	-	309.4	304.5	272.5	291.8	268.3	239.0	279.9	270.1	-	257.8
430	275.6	253.4	287.6	277.5	252.2	267.6	283.9	220.1	277.1	249.7	205.5	236.6	251.0	253.7
431	276.6	236.1	255.8	264.9	219.6	249.3	253.6	-	229.1	275.7	268.2	172.4	170.5	156.1
SAMPLE	4	3	4	3	4	4	4	3	4	4	4	4	3	4
MEAN	244.6	210.5	266.8	249.9	245.6	259.3	264.1	241.9	243.2	231.5	243.0	220.6	200.7	219.5
S.D.	29.4	26.2	24.1	31.4	47.5	37.1	17.2	43.3	36.4	49.2	36.5	42.1	43.9	47.4
S.E.	14.7	13.1	12.0	21.6	23.8	18.5	8.6	25.0	18.2	24.6	18.3	21.1	25.3	23.7

PROJECT NO. 107340V
COMPUTER GROUP NO: 301E
LITTON BIONNETICS, INC.
DAILY FOOD INTAKE IN GRAMS
GROUP 3 MALES
DOSE: 300 PPM
TABLE 3 (CONTINUED)

TABLE 3 (CONTINUED)														
ANML NO.	DATES OF TESTING (1978)													
	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	8/16
404	215.5	266.0	274.6	335.1	294.1	306.6	328.4	267.4	275.3	323.8	313.9	298.9	292.7	319.6
409	342.0	277.4	305.8	302.5	331.2	289.5	258.1	227.9	—	—	284.2	271.2	270.4	336.1
412	239.5	248.0	264.6	—	220.2	222.0	325.2	228.9	251.3	—	—	188.5	337.5	190.7
414	—	308.1	319.3	300.9	286.2	—	304.1	—	—	275.7	315.4	291.6	306.6	244.7
SAMPLE	3	4	4	3	4	3	4	3	2	2	3	4	4	4
MEAN	265.6	276.9	291.6	312.4	286.9	272.7	303.9	261.4	263.3	299.7	304.5	262.5	301.8	272.8
S.D.	67.2	25.3	40.0	19.3	42.6	44.8	32.4	22.5	17.6	34.0	17.6	50.8	28.1	67.6
S.E.	38.8	12.6	20.0	11.1	21.3	25.8	16.2	13.0	12.0	24.0	10.2	25.4	14.0	33.8

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TABLE II-F-46 (Continued)

PROJECT HU. 10/3409
 COMPUTER GROUP NO: 3011
 LITTON BIONETICS, INC.
 DAILY FOOD INTAKE IN GRAMS
 GROUP 3 FEMALES
 DOSE: 300 PPM

TABLE 3 (CONTINUED)

ANML NO.	5/17	5/24	5/31	6/7	6/14	6/21	6/28	7/5	7/12	7/19	7/26	8/2	8/9	8/16
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
421	-	273.5	352.7	-	313.3	-	318.7	277.6	316.8	275.2	270.6	307.8	276.8	303.9
424	232.3	276.9	289.0	245.1	205.8	219.1	305.5	247.8	-	-	278.7	246.6	292.4	273.1
427	244.4	221.5	244.5	231.1	196.1	207.9	224.7	196.3	-	-	205.6	204.4	211.7	214.1
433	330.5	-	-	-	-	262.7	343.6	-	-	-	-	-	-	-
SAMPLE	3	3	3	2	3	3	4	3	1	1	3	3	3	3
MEAN	269.1	277.3	299.7	230.1	238.6	229.9	290.1	227.2	316.8	275.2	251.6	252.9	260.3	263.7
S.D.	53.6	31.0	54.9	9.9	66.8	28.9	51.4	63.2	0.0	0.0	40.1	52.0	42.8	45.7
S.E.	30.9	17.9	31.7	7.0	37.4	16.1	25.1	36.5	0.0	0.0	23.2	30.0	24.7	26.4

TABLE II-F-46 (Continued)

PROJECT NO. 1073409														
COMPUTER GROUP NO: 3011.														
LITTON BIONETICS, INC.														
DAILY FOOD INTAKE IN GRAMS														
GROUP 4 MALES														
DUE1: 1000 PPM														
TABLE 3 (CONTINUED)														
ANAL DATES OF TESTING (1978)														
NO.	5/17	5/24	5/31	6/ 7	6/14	6/21	6/28	7/ 5	7/12	7/19	7/26	8/ 2	8/ 9	8/16
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
413	-	301.8	201.3	376.4	222.1	324.2	-	-	330.1	321.0	294.3	-	224.5	292.3
416	-	402.3	264.7	-	266.6	242.3	274.8	260.0	323.3	271.5	306.3	341.7	261.6	282.2
419	317.9	293.6	293.1	267.2	324.5	280.1	308.7	250.8	248.6	272.9	200.8	-	-	339.7
420	339.8	-	411.9	442.1	-	-	-	-	260.6	-	354.5	406.8	-	358.1
SAMPLE	2	3	4	3	3	3	2	2	4	3	4	2	2	4
MEAN	428.9	332.6	292.8	362.9	271.1	282.2	291.8	255.4	290.7	288.5	289.0	374.2	243.1	318.1
S.D.	15.4	60.5	88.2	88.6	51.4	41.0	24.0	6.5	42.0	28.2	64.3	46.0	26.3	36.6
S.E.	10.9	34.9	44.1	51.2	29.7	23.7	17.0	4.6	21.0	16.3	32.1	32.5	18.6	18.3

TABLE II-F-46 (Continued)

PROJECT NO. 10/3409 COMPUTER GROUP NO. 3011 LITTON BIONETICS, INC. DAILY FOOD INTAKE IN GRAMS GROUP 4 FEMALES TABLE 3 (CONTINUED) ANAL DATES OF TESTING (1978) NO. 5/17 5/24 5/31 6/7 6/14 6/21 6/28 7/5 7/12 7/19 7/26 8/2 8/9 8/16															
423	273.4	—	288.4	232.4	199.1	—	260.1	—	253.8	188.6	236.3	—	—	307.5	14
432	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.0
436	181.3	205.2	202.0	359.2	195.1	220.3	—	204.4	200.9	157.9	181.8	248.7	262.1	177.0	14
437	216.9	233.8	247.9	273.0	276.2	244.8	244.1	218.4	232.4	252.5	250.2	277.5	228.8	251.5	14
SAMPLE	J	2	3	3	J	2	2	2	3	3	3	2	2	4	
MEAN	223.8	219.5	246.1	288.2	222.8	232.6	252.1	241.4	229.0	199.7	222.7	263.1	245.5	184.0	
S.D.	46.4	20.2	43.2	64.8	44.6	17.4	11.3	32.5	26.6	48.2	36.2	20.4	23.5	133.8	
S.F.	26.8	14.3	25.0	37.4	25.7	12.3	8.0	23.0	15.4	27.9	20.9	14.4	16.7	66.9	

* p<0.05 as compared to controls: Dunnett's t-test.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 4

CLINICAL HEMATOLOGY

KEY

WBC	= Leukocyte Count	$10^3/\text{mm}^3$
RBC	= Erythrocyte Count	$10^6/\text{mm}^3$
HGB	= Hemoglobin	g%
HCT	= Hematocrit	vol %
BN	= Band Neutrophils	%
SG	= Segmented Neutrophils	%
LY	= Lymphocytes	%
MO	= Monocytes	%
EO	= Eosinophils	%
BS	= Basophils	%
OT	= Other	%
R	= Repeat value	
S	= Repeat attempted without success, first value taken	

TABLE II-F-47

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4
INDIVIDUAL ANIMAL HEMATOLOGIES--MALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	PN	SG	LY	MO	CU	US	OT
CONTROL	408	9.9	6.03	14.8	44.0	.	27	68	.	5	.	.
	411	10.1	6.03	14.9	42.0	.	34	65	1	.	.	.
	417	9.1	6.22	13.0	38.5	.	33	62	1	4	.	.
	418	9.0	6.20	14.8	43.0	.	58	41	.	1	.	2
	MEAN	9.52	6.120	14.37	41.38							
	SE	0.28	0.052	0.46	1.20							
	N	4	4	4	4							
100 PPM	405	15.4	6.94	15.7	46.5	.	38	57	3	1	.	.
	406	10.7	6.79	16.4	47.0	.	35	63	2	.	.	.
	407	8.6	6.42	15.3	40.0	.	25	40	1	6	.	.
	415	10.1	7.12	15.4	44.5	.	41	53	1	.	.	.
	MEAN	11.20	6.817	15.70	46.00							
	SE	1.47	0.149	0.25	0.54							
	N	4	4	4	4							
300 PPM	404	7.7	7.38	16.8	49.0	.	16	83	1	.	.	.
	409	7.6	6.98	15.1	44.5	.	40	54	3	3	.	.
	412	10.2	6.59	16.9	48.5	.	48	51	1	.	.	.
	414	11.0	6.21	14.3	43.5	.	61	39
	MEAN	9.12	6.790	15.77	46.36							
	SE	0.87	0.252	0.64	1.39							
	N	4	4	4	4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES--MALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	SG	LY	PL	EO	OS	OT
1000 PPM	413	11.8	5.61	14.7	42.0	28	60	2	2	2	.
	416	12.0	6.53	15.1	44.5	55	34	3	8	.	.
	419	9.9	6.81	15.3	47.0	44	55	.	1	1	11
	420	9.7	6.90	15.7	45.0	13	86	1	.	.	8
MEAN SE N		10.85	6.462	15.20	44.63						
		0.61	0.295	0.21	1.03						
		4	4	4	4						

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-FEMALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	DN	SG	LY	MU	LU	HS	GT
CONTROL	425	10.1	7.90	17.0	50.5	.	31	65	.	2	.	1
	426	7.7	7.23	10.2	44.0	.	61	35	.	4	.	1
	434	14.9	6.76	14.9	43.0	1	39	60
	435	8.6	6.91	17.0	50.0	.	38	62
	MEAN SE N	10.32 1.60 4	7.200 0.253 4	16.77 0.69 4	49.38 2.30 4							
100 PPM	422	10.7	7.36	15.4	46.5	.	60	36	2	1	1	.
	429	10.6	6.81	17.8	51.0	.	37	59	3	1	.	.
	430	11.2	6.14	14.9	44.5	.	26	74
	431	9.2	6.27	16.3	47.5	.	46	51	1	2	0	.
	MEAN SE N	10.42 0.43 4	6.645 0.279 4	16.10 0.64 4	46.48 1.55 4							
300 PPM	421	11.3	6.92	16.5	48.5	.	22	76	.	2	.	19
	424	9.8	6.59	15.1	44.0	.	35	63	2	.	.	4
	427	8.8	7.39	17.0	53.0	.	56	42	1	1	.	4
	433	9.1	6.08	16.3	47.5	.	35	55	3	7	.	.
	MEAN SE N	9.75 0.56 4	6.945 0.166 4	16.42 0.55 4	48.25 1.85 4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-FEMALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	UN	SG	LY	MC	LU	US	OT
1000 PPM	423	10.0	6.89	15.9	46.5	.	34	60	.	0	.	.
	432	7.8	6.64	16.2	47.5	.	66	30	2	2	.	10
	436	8.6	6.48	16.4	49.0	.	33	65	1	1	.	13
	437	8.4	7.63	17.2	51.5	1	55	43	1	.	.	.
	MEAN	8.70	6.910	16.42	48.63							
	SE	0.47	0.254	0.28	1.09							
	N	4	4	4	4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FCA P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES--WEEK 4
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	UN	SG	LY	MO	EO	BS	OT
CONTROL	408	10.6	7.03	15.5	42.5	•	41	44	6	9	•	3
	411	10.6	6.27	15.1	45.0	•	57	41	•	2	•	•
	417	8.7	7.15	15.0	41.0	•	45	51	2	2	•	•
	418	11.9 R	7.27 F	16.1 R	46.5 R	•	54	40	1	5	•	•
100 PPM	MEAN	10.45	6.920	15.42	43.75							
	SE	0.66	0.225	0.25	1.23							
	N	4	4	4	4							
	405	12.0	6.72	15.3	45.0	•	63	26	•	1	•	•
300 PPM	406	8.1	6.84	16.2	45.0	•	26	72	•	2	•	•
	407	9.9	8.37	16.4	46.0	•	42	47	6	5	•	2
	415	11.6	7.63	15.1	45.0	•	40	44	3	4	1	•
	MEAN	10.40	7.390	15.75	45.25							
300 PPM	SE	0.89	0.384	0.32	0.25							
	N	4	4	4	4							
	404	9.3	6.87	15.9	45.0	•	38	60	2	•	•	•
	409	8.6	6.83	15.8	44.0	•	30	67	2	•	1	•
300 PPM	412	10.8	6.81	15.7	44.5	•	62	25	5	7	1	•
	414	13.8	8.45	16.6	43.0	•	37	19	4	2	1	•
	MEAN	10.62	7.240	16.00	44.13							
	SE	1.15	0.404	0.20	0.43							
	N	4	4	4	4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-HALFS
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	DP: SG	LY	MO	EO	HS	OT
1000 PPM	413	11.5	6.40	15.9	45.0	2	42	36	7	13	.
	416	13.1 K	7.18 R	15.9 K	46.0 Q	.	56	31	2	11	.
	419	9.4	6.42	15.3	43.0	.	19	78	1	2	.
	420	9.6	6.18	14.6	41.0	3	42	52	2	1	.
MEAN SE N:		10.90	6.545	15.42	43.75						
		0.87	0.215	0.31	1.11						
		4	4	4	4						

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGICAL VALUES
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	INT	SG	LY	MO	EO	HS	OT
CONTROL	425	9.5	8.36	16.6	49.0	5	57	26	7	7	.	1
	426	9.6	6.58	15.0	49.0	.	55	36	2	5	1	5
	434	9.0	7.15	15.7	45.0	.	28	69	.	3	.	.
	435	8.8	7.30	17.7	50.0	.	65	33	1	1	.	.
100 PP4	MEAN	9.22	7.447	16.47	48.25							
	SE	0.19	0.311	0.45	1.11							
	N	4	4	4	4							
	422	11.6	6.81	15.5	44.0	2	62	20	12	4	.	.
300 PP4	429	11.0	7.62	18.9	52.0	.	45	54	.	.	1	.
	430	9.7	7.23	16.1	45.0	1	56	34	6	3	.	2
	431	10.9	7.33	15.8	44.0	.	45	52	2	1	.	.
	MEAN	10.80	7.247	16.57	46.25							
300 PP4	SE	0.40	0.168	0.78	1.93							
	N	4	4	4	4							
	421	12.6	7.47	17.8	50.0	1	51	45	.	2	1	.
	424	11.5	6.84	16.0	47.0	2	66	21	6	5	.	2
300 PP4	427	12.9	6.80	15.6	48.0	1	67	25	4	2	1	.
	433	15.3	6.28	15.2	42.0	.	47	46	1	5	1	.
	MEAN	13.07	6.847	16.15	46.75							
	SE	0.80	0.244	0.57	1.70							
	N	4	4	4	4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-FEMALES
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO	WBC	PBC	HGB	HCT	MM	SG	LY	MO	EO	BS	UT
1000 PPM	423	9.2	6.96	16.2	45.0	•	27	71	2	•	•	•
	432	18.0	6.85	15.8	46.0	•	67	23	6	4	•	•
	436	11.7	6.98	16.2	46.5	1	55	36	2	6	•	2
	437	11.5	7.10	17.7	49.0	4	25	67	1	3	•	•
MEAN SE N		12.60 1.89 4	6.972 0.051 4	16.47 0.42 4	46.63 0.85 4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073499

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGICAL VALUES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	BM	SS	LY	MO	EO	PS	GT
CONTROL	408	11.3 K	6.46 P	14.5 P	46.0 P	•	47	43	•	10	•	•
	411	10.7	5.88	15.2	44.5	•	68	29	1	2	•	•
	417	6.5	5.47	12.0	38.0	•	52	26	5	16	1	•
	418	15.2	6.50	14.0	43.0	•	66	16	11	5	1	•
	MEAN SE N	10.92 1.78 4	6.077 0.247 4	13.92 0.60 4	42.86 1.74 4							
100 PPM	405	13.6	7.07	15.2	45.0	•	52	25	7	16	•	1
	406	8.5	7.19	16.0	47.0	•	1	53	41	3	2	•
	407	9.0	5.93	14.4	44.0	•	2	54	30	3	11	•
	415	9.9	7.19	15.5	48.0	•	1	50	38	4	7	•
	MEAN SE N	10.25 1.15 4	6.815 0.306 4	15.27 0.34 4	46.00 0.91 4							
300 PPM	404	9.9 R	7.26 P	14.6 R	45.0 K	•	2	27	67	1	3	•
	409	9.1 S	6.67 S	15.0 S	45.0 S	•	58	33	1	8	•	•
	412	10.3	6.07	15.4	45.0	•	60	26	6	7	1	•
	414	14.0	5.84	14.1	44.0	•	63	30	6	1	•	•
	MEAN SE N	10.82 1.00 4	6.420 0.310 4	14.77 0.28 4	44.75 0.25 4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-107360

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-MALES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	WBC	PCV	HGB	HCT	RW	SG	LY	MO	EO	HS	NT
1000 PPH	412	12.8	6.47	14.4	44.0	2	48	30	9	10	1	.
	416	12.2	6.64	14.3	44.0	.	37	37	1	22	3	.
	419	8.4	7.14	14.8	46.0	.	64	19	9	8	.	.
	420	7.5	6.50	14.2	43.0	.	17	25	4	4	.	2
MEAN SF N	10.22	6.687	14.42	44.25	4							
	1.53	0.155	0.13	0.63								

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-107340C

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGICS-FEMALES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	DN	SG	LY	MO	EO	HS	BT
CONTROL	425	11.9	6.54	14.6	44.0	.	64	24	3	9	.	1
	426	10.5	7.10	15.6	50.0	.	81	12	1	3	3	.
	434	7.3	6.25	13.9	41.0	.	61	23	9	5	2	1
	435	7.4	6.35	15.8	48.0	.	58	24	4	14	.	.
	MEAN SF N	9.27 1.15 4	6.58 0.191 4	15.22 0.60 4	45.75 2.02 4							
100 PPM	422	7.6	7.22	13.9	40.5	.	40	46	6	8	.	1
	429	10.6	6.67	15.8	46.0	1	64	22	4	9	.	3
	430	10.7	6.95	14.9	47.5	.	60	29	8	3	.	.
	431	12.7	6.14	14.0	42.0	1	55	34	10	.	.	1
	MEAN SF N	10.40 1.05 4	6.530 0.265 4	14.65 0.44 4	44.00 1.65 4							
300 PPM	421	13.3	6.92	17.0	50.0	.	59	34	3	2	2	2
	424	10.0	7.08	16.3	47.5	.	58	29	2	9	2	1
	427	8.4	6.55	16.6	47.0	.	63	25	5	4	3	1
	423	13.4	6.52	16.4	48.0	.	58	25	8	3	.	2
	MEAN SF N	11.27 1.24 4	6.877 0.103 4	16.57 0.15 4	48.13 0.65 4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-FEMALES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	III	SG	LY	MC	EO	BS	OT
1000 PPM	423	11.2	6.04	12.9	35.0	1	50	35	3	8	3	.
	432	13.7	5.97	14.6	46.0	.	67	19	8	6	.	1
	436	9.4	6.53	15.1	41.5	.	61	29	9	1	.	1
	437	9.0	7.22	17.1	50.0	.	38	51	4	7	.	3
MEAN SF N	MEAN	10.82	6.440	14.02	44.63							
	SF	1.07	0.208	0.86	2.30							
	N	4	4	4	4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073409

TABLE 4 (CONTINUED) HEMATOLOGIES-MALES
INDIVIDUAL ANIMALS
INTERVAL OF STUDY = TERMINAL KILL

DOSE GROUP	ANIMAL NO	WBC	PMN	HGB	HCT	HP: SG LY MO CO BS OT
CONTROL	408	10.9	7.43	16.2	44.0	• 54 35 4 3 • • •
	411	8.6	6.71	16.3	46.0	• 47 30 2 12 • • •
	417	8.1	5.87	12.9	37.0	• 5 64 20 3 7 1 • •
	418	10.9	7.05	15.6	44.0	• 4 57 31 3 5 • •
100 PP4	MEAN	9.62	6.765	15.25	42.75	
	SE	0.74	0.333	0.80	1.57	
	N	4	4	4	4	
100 PP4	405	13.7	7.36	16.6	47.0	• 6 71 12 2 9 • • •
	406	8.4	7.72	17.2	49.0	• 2 57 30 8 3 • • •
	407	8.8	7.03	15.7	45.0	• • 39 49 • 10 1 6 • •
	415	14.8	6.52	15.7	44.0	• 5 65 26 1 3 • • •
300 PP4	MEAN	11.45	7.157	16.30	46.25	
	SE	1.67	0.255	0.27	1.11	
	N	4	4	4	4	
300 PP4	404	8.0	6.80	14.8	43.5	• 63 27 5 4 • 11 •
	409	9.6	7.24	16.7	47.0	• 46 46 1 7 • 13 •
	412	7.5	7.30	17.4	49.0	• 52 41 • 7 • • •
	414	12.4	6.53	15.1	43.0	• 74 20 • 1 • • •
300 PP4	MEAN	9.37	6.967	16.00	45.63	
	SE	1.10	0.184	0.63	1.43	
	N	4	4	4	4	

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1071400

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES--MALLS
INTERVAL OF STUDY--TERMINAL KILL

DOSE GROUP	ANIMAL NO	WBC	PLC	HGB	HCT	MCV	MC	PC	MS	OT
1000 PPM	413	11.6	7.12	14.5	43.0	54	34	4	8	12
	416	9.5	7.27	15.1	44.5	59	32	1	6	75
	419	8.1	6.66	15.8	45.0	63	28	1	7	1
	420	8.7	6.38	14.8	42.0	58	37	2	5	8
MEAN ST N		9.47	6.857	15.05	43.63					
		0.76	0.705	0.28	0.65					
		4	4	4	4					

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1073439

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-FEMALE
INTERVAL OF STUDY- TERMINAL KILL

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	HA	SG	LY	MO	EO	NS	LT
CONTROL	425	10.3	6.39	15.4	44.0	1	53	36	5	5	1	
	426	10.9	7.22	17.0	48.0	1	63	20	4	12	1	
	434	9.8	6.83	14.1	40.0	1	51	39	3	7	13	
	435	9.4	7.76	17.2	49.0	1	68	46	1	5	1	
	MEAN	10.10	7.175	15.92	45.25							
	SE	0.32	0.213	0.73	2.05							
	N	4	4	4	4							
100 PPM	422	11.3	6.13	13.6	37.0	2	34	54	10	17	1	
	429	9.7	6.25	16.0	45.0	1	67	28	3	2	1	
	430	9.3	6.27	17.4	50.0	1	44	44	6	3	11	
	431	7.0	6.83	16.9	47.0	1	35	62	2	1	1	
	MEAN	9.37	6.870	15.97	44.75							
	SE	0.90	0.461	0.84	2.78							
	N	4	4	4	4							
300 PPM	421	14.0	8.52	17.0	49.0	3	63	28	2	4	1	
	424	11.8	6.71	16.1	45.0	2	64	27	7	1	19	
	427	8.1	6.20	14.9	41.5	2	64	23	11	1	4	
	433	15.1	8.02	16.2	46.3	4	56	17	5	14	2	11
	MEAN	12.52	7.365	14.05	45.13							
	SE	1.67	0.545	0.43	1.36							
	N	4	4	4	4							

TABLE II-F-47 (Continued)

CLINICAL HEMATOLOGIES FOR P-1072409

TABLE 4 (CONTINUED)
INDIVIDUAL ANIMAL HEMATOLOGIES-15 HALFS
INTERVAL OF STUDY= TERMINAL KILL

DOSE GROUP	ANIMAL NO	WBC	RBC	HGB	HCT	BMF SG LY MG LS HS CT									
1000 PPM	423	10.6	7.05	15.8	45.0	•	49	38	4	9	•	•	5	•	•
	422	12.6	6.77	15.6	47.0	•	67	31	1	1	•	•	16	•	•
	436	9.2	5.87	13.0	40.0	•	2	60	30	3	5	•	•	13	•
	427	9.6	8.00	19.2	59.0	•	3	41	45	2	9	•	•	•	•
MEAN SE N		10.45	6.922	16.20	46.75										
		0.70	0.439	1.10	3.12										
		4	4	4	4										

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 5

CLINICAL CHEMISTRY

KEY

BILI	= Bilirubin, total	mg/dl
BUN	= Blood Urea Nitrogen	mg/dl
ALB	= Albumin	g/dl
CA	= Calcium	mg/dl
CHOL	= Cholesterol	mg/dl
GLCS	= Glucose	mg/dl
LDH	= Lactic Dehydrogenase	mU/ml
SAP	= Alkaline Phosphatase	mU/ml
PHOS	= Phosphorus	mg/dl
PROT	= Protein, total	g/dl
SGOT	= Serum Glutamic-oxaloacetic Transaminase	mU/ml
SGPT	= Serum Glutamic-pyruvic Transaminase	mU/ml
URIC	= Uric Acid	mg/dl
NA	= Sodium	meg/l
K	= Potassium	meg/l
CL	= Chloride	meg/l
A	= Test not performed	
R	= Repeat value	
S	= Repeat attempted without success, first value taken	
*	= $p < 0.05$ as compared to controls: Dunnett's t-test	

TABLE II-F-48

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5
INDIVIDUAL ANIMAL CHEMISTRIES-FEMALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC	NA	K	CL
CONTROL	425	0.1	15	3.3	11.1	145	70	96	62	4.1	6.0	28	22	0.2	147	4.6	108
	426	0.1	11	4.6	11.3	155	88	204	61	3.5	6.6	33	48	0.4	148	4.3	110
	434	0.1	14	3.6	11.0	135	85	166	34	4.7	6.1	29	47	0.3	149	4.8	109
	435	0.1	13	4.7	10.9	145	116	165	38	3.1	6.1	27	7	0.4	148	4.8	111
	MEAN	0.10	13.3	4.05	11.07	145.0	89.8	157.8	48.8	3.85	6.20	29.3	31.0	0.32	148.0	4.62	109.5
	SE	0.00	0.9	0.25	0.09	4.1	9.6	22.5	7.4	0.35	0.14	1.3	10.0	0.05	0.4	0.12	0.6
	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
100 PPM	422	0.1	11	3.1	11.1	140	73	56	68	4.8	5.8	26	44	0.2	146	4.8	108
	429	0.1	12	3.5	11.0	138	66	55	68	4.7	6.1	28	30	0.2	146	4.5	108
	430	0.1 R	15	3.3 R	11.4 R	134 R	101 R	175 R	46 R	4.3 R	6.1 R	37 R	45 P	0.3 R	148 R	5.7 R	108 R
	431	0.1	16	3.8	11.0	125	82	110	63	4.6	6.1	34	43	0.3	148	4.7	108
	MEAN	0.10	13.5	3.42	11.12	134.3	80.5	99.0	61.3	4.60	6.02	31.3	40.5	0.25	147.0	4.92	108.0
	SE	0.00	1.2	0.15	0.09	3.3	7.5	28.4	5.2	0.11	0.08	2.6	3.5	0.03	0.6	0.27	0.0
	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
300 PPM	421	0.1	20	4.5	11.3	185	100	160	61	3.8	6.2	39	34	0.5	148	4.4	107
	424	0.1	12	3.3	10.5	136	78	152	69	4.3	6.0	31	41	0.3	148	4.1	109
	427	0.1 R	10	3.1 R	11.2 R	153 R	85 R	250 F	47 R	3.9 R	6.7 R	25 R	28 R	0.2 R	147 R	5.3 R	110 R
	433	0.1	15	4.2	10.7	156	100	146	73	3.9	5.9	27	33	0.1	145	4.8	108
	MEAN	0.10	14.3	3.77	10.92	157.5	90.8	177.0	62.5	3.97	6.20	30.5	34.0	0.27	147.0	4.65	108.5
	SE	0.00	2.2	0.34	0.19	10.2	5.5	24.5	5.7	0.11	0.18	3.1	2.7	0.09	0.7	0.26	0.6
	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-FEMALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC	NA	K	CL
1000 PPM	423	0.0	20	4.8	11.7	171	101	280	59	5.4	6.4	40	32	0.5	152	5.1	112
	432	0.1	12	3.1	10.1	133	69	142	70	5.8	5.3	34	38	0.4	145	5.0	108
	436	0.1	10	4.6	11.0	126	92	110	84	4.5	6.1	27	53	0.4	147	4.8	110
	437	0.1	12	3.6	11.0	157	72	50	64	5.1	5.9	26	50	0.1	145	5.0	109
MEAN SE N		0.07	13.5	4.02	10.95	146.8	83.5	145.5	69.3	5.20	5.92	31.8	43.3	0.35	147.3	4.97	109.8
		0.02	2.2	0.40	0.33	10.5	7.8	48.7	5.4	0.27	0.23	3.3	5.0	0.09	1.7	0.06	0.5
		4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-MALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC	HA	K	CL
CONTROL	408	0.1 R	10 R	3.2 R	10.8 R	136 R	97 R	192 F	94 R	5.2 R	6.1 R	37 R	40 R	0.4 R	145 R	5.2 R	108 R
	411	0.0	16	4.4	11.6	151	90	239	62	5.0	6.9	31	25	0.4	147	5.3	105
	417	0.1 R	14 R	3.0 R	10.7 R	144 R	97 R	546 P	66 R	5.1 R	5.5 R	37 R	31 R	0.3 R	147 R	5.3 R	109 R
	418	0.1	14	4.6	11.4	163	102	151	81	4.3	6.5	34	54	0.5	150	4.6	112
100 PPM	MEAN	0.07	13.5	3.80	11.12	148.5	96.5	282.0	75.8	4.90	6.25	34.8	37.5	0.40	147.3	5.10	108.5
	SE	0.02	1.3	0.41	0.22	5.7	2.5	89.8	7.3	0.20	0.30	1.4	6.3	0.04	1.0	0.17	1.4
	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	405	0.0	18	4.0	11.4	148	91	240	162	5.3	6.7	31	39	0.4	147	4.8	106
300 PPM	406	0.0	18	4.7	11.7	184	87	93	63	5.2	6.6	26	31	0.4	147	4.9	108
	407	0.1 R	14 R	3.3 R	10.8 R	172 R	87 R	164 R	62 R	4.6 R	6.2 R	33 R	31 R	0.4 R	144 R	4.9 R	108 R
	415	0.1	13	3.1	10.6	145	72	82	65	5.2	5.8	36	49	0.4	147	4.2	110
	MEAN	0.05	15.8	3.77	11.12	162.3	84.3	144.8	88.0	5.07	6.32	31.5	37.5	0.40	146.3	4.70	106.0
300 PPM	SE	0.03	1.3	0.36	0.26	9.4	4.2	36.6	24.7	0.16	0.21	2.1	4.3	0.00	0.8	0.17	0.8
	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	404	0.1	12	4.7	11.4	180	101	188	67	4.4	6.7	31	36	0.4	147	4.7	108
	409	0.1	11	3.3	10.7	144	72	123	61	4.9	6.3	30	38	0.4	143	4.8	108
300 PPM	412	0.0	10	4.7	11.2	193	92	262	69	3.5	6.4	32	47	0.5	146	4.9	108
	414	0.1	12	4.1	11.0	176	97	94	79	5.1	6.8	29	35	0.5	144	4.9	106
	MEAN	0.07	11.5	4.20	11.07	174.0	90.5	166.8	69.0	4.47	6.55	30.5	39.0	0.45	145.0	4.82	107.5
	SE	0.02	0.6	0.33	0.15	10.5	6.4	37.3	3.7	0.36	0.12	0.6	2.7	0.03	0.5	0.05	0.5
	N	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-MALES
INTERVAL OF STUDY= INITIAL

DOSE GROUP	ANIMAL NC	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC	NA	K	CL
1000 PPM	413	0.1	11	3.3	10.7	144	81	68	145	4.1	5.8	28	34	0.5	145	4.4	108
	416	0.1	16	4.5	11.0	120	100	174	52	4.0	5.8	34	53	0.6	148	4.7	109
	419	0.0	14	4.2	11.2	150	91	244	77	4.7	6.2	41	60	0.4	146	4.9	107
	420	0.1 R	B P	3.3 R	11.0 R	149 R	92 R	170 F	67 R	4.8 R	6.0 R	32 R	76 R	0.3 R	146 R	5.0 R	113 R
MEAN	SE	0.07	12.3	3.82	10.97	140.8	91.0	164.0	85.3	4.40	5.95	33.8	55.8	0.45	146.3	4.75	109.3
		0.02	1.8	0.31	0.10	7.0	3.9	36.2	20.6	0.20	0.10	2.7	8.7	0.06	0.6	0.13	1.3
		N		4	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-MALES
INTERVAL OF STUDY WEEK 4

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LOH	SAP	PHOS	PROT	SGOT	SGPT	URIC
CONTROL	408	0.1	14	3.2	10.5	131	89	123	83	4.9	6.5	72	52	0.4
	411	0.1	20	3.2	11.6	155	78	463	55	4.5	6.8	40	29	0.2
	417	0.1	14	2.8	10.4	121	87	332	57	4.1	5.5	39	34	0.1
	418	0.1	14	3.2	10.8	134	90	243	37	4.1	6.0	66	39	0.5
	MEAN	0.10	15.5	3.10	10.82	135.3	86.0	290.3	58.0	4.40	6.20	54.3	38.5	0.30
	SE	0.00	1.5	0.10	0.27	7.1	2.7	71.8	9.5	0.19	0.29	8.6	4.9	0.09
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
100 PPM	405	0.0	19	3.0	11.1	152	81	351	105	4.2	6.6	45	44	0.0
	406	0.1	15	3.0	11.3	176	81	205	45	4.4	6.4	32	27	0.0
	407	0.1	15	3.0	10.6	187	92	174	55	4.6	6.1	106	32	0.4
	415	0.1	17	3.1	10.7	143	80	299	54	3.6	5.8	72	32	0.3
	MEAN	0.07	16.5	3.02	10.92	164.5	83.5	257.3	64.8	4.20	6.22	63.8	33.8	0.17
	SE	0.02	1.0	0.02	0.17	10.2	2.8	41.0	13.6	0.22	0.18	16.4	3.6	0.10
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
300 PPM	404	0.1	13	2.7	10.5	156	81	279	56	4.0	6.2	40	32	0.0
	409	0.1	16	2.6	10.6	121	86	284	58	4.4	6.1	38	39	0.0
	412	0.1	16	3.2	10.8	154	82	134	56	4.1	5.6	47	42	0.4
	414	0.1	16	3.1	11.2	165	89	129	76	4.7	6.9	80	28	0.4
	MEAN	0.10	15.3	2.90	10.77	149.0	84.5	206.5	61.5	4.30	6.20	51.3	35.3	0.20
	SE	0.00	0.8	0.15	0.15	9.6	1.8	43.3	4.9	0.16	0.27	9.8	3.2	0.12
	N	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES—MALES
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO	BIL I	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
1000 PPM	413	0.1	14	3.0	10.4	148	88	247	137	3.8	5.9	67	26	0.6
	416	0.1	14	2.9	10.7	157	98	95	92	4.1	6.3	51	39	0.4
	419	0.1	15	2.7	10.8	137	80	286	56	4.4	6.2	40	42	0.9
	420	0.1	18	2.9	10.6	132	90	298	65	4.2	6.1	56	62	0.3
MEAN SE N	0.10	15.3	2.87	10.62	143.5	89.0	231.5	87.5	4.12	5.5	42.3	0.55		
	0.00	0.9	0.06	0.09	5.6	3.7	46.8	18.2	0.13	5.6	7.4	0.13		
	4	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-FEMALES
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO.	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
CONTROL	425	0.1	12	3.6	11.0	138	84	164	55	3.6	5.9	65	28	0.4
	426	0.2	13	3.3	10.9	127	81	124	52	4.0	6.3	67	37	0.3
	434	0.1	13	3.0	11.0	114	81	975	32	3.9	6.3	48	32	0.1
	435	0.1	12	3.2	10.9	138	88	483	31	3.2	6.0	49	39	0.0
	MEAN	0.12	12.5	3.27	10.95	129.3	83.5	436.5	42.5	3.67	6.12	57.3	34.0	0.20
	SE	0.02	0.3	0.12	0.03	5.7	1.7	196.7	6.4	0.18	0.10	5.1	2.5	0.09
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
100 PPM	422	0.1	10	2.9	11.1	128	86	117	87	4.7	5.6	55	31	0.4
	429	0.1	11	3.2	10.9	165	75	371	81	3.2	6.4	43	27	0.0
	430	0.1	18	3.3	11.3	131	83	316	43	4.2	5.8	87	34	0.4
	431	0.1	19	3.4	11.1	113	93	355	50	4.0	6.1	37	33	0.1
	MEAN	0.10	14.5	3.20	11.10	134.3	84.3	289.8	65.3	4.02	5.97	55.5	31.3	0.22
	SE	0.00	2.3	0.11	0.08	11.0	3.7	58.7	11.0	0.31	0.18	11.1	1.5	0.10
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
300 PPM	421	0.1	26	2.9	11.0	197	79	320	64	3.6	6.2	48	40	0.3
	424	0.1	13	3.1	10.7	146	87	127	72	5.0	5.9	69	79	0.5
	427	0.1	11	3.3	11.1	183	81	261	44	3.4	6.6	71	24	0.3
	433	0.1	14	1.4	10.5	163	85	231	82	4.4	6.0	36	27	0.5
	MEAN	0.10	16.0	2.67	10.82	172.3	83.0	234.8	65.5	4.10	6.17	56.0	42.5	0.40
	SE	0.00	3.4	0.43	0.14	11.2	1.8	40.4	8.1	0.37	0.15	11.1	12.7	0.06
	N	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES--FEMALES
INTERVAL OF STUDY= WEEK 4

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
1000 PPM	423	0.1	25	3.2	11.7	231	80	473	54	4.4	6.7	45	23	0.4
	432	0.1	16	2.8	10.2	112	76	222	80	4.7	5.5	72	36	0.5
	436	0.1	11	3.2	10.7	134	82	213	72	3.8	6.0	68	29	0.3
	437	0.1	15	2.9	11.4	176	72	479	70	3.6	6.0	50	35	0.3
MEAN SF N		0.10	16.8	3.02	11.00	163.3	77.5	346.8	69.0	4.12	6.05	58.8	30.8	0.37
		0.00	3.0	0.10	0.34	26.2	2.2	74.7	5.4	0.26	0.25	6.6	3.0	0.05
		4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-MALES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	BILI	BUN	A-B	CA	CHOL	GLCS	LOH	SAP	PHOS	PROT	SGOT	SGPT	URIC
CONTROL	408	0.1	14	3.3	11.1	131	90	97	61	4.7	6.6	54	82	0.1
	411	0.1	19	3.1	11.1	152	87	257	47	4.1	6.7	34	25	0.3
	417	0.0	17	2.9	10.1	120	95	227	59	4.3	5.4	37	33	0.2
	418	0.1	11	3.2	11.2	143	83	56	66	4.0	6.4	25	46	0.0
100 PPM	MEAN	0.07	15.3	3.12	10.87	136.5	88.8	166.8	58.3	4.27	6.27	37.5	46.5	0.15
	SE	0.02	1.8	0.09	0.26	7.0	2.5	43.9	4.0	0.15	0.30	6.1	12.6	0.06
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
100 PPM	405	0.0	18	3.0	10.8	123	94	139	78	4.4	6.2	35	41	0.0
	406	0.0	15	3.4	11.6	166	84	170	36	4.5	6.4	37	26	0.0
	407	0.1	16	3.0	10.5	173	96	111	46	4.4	6.1	29	33	0.2
	415	0.1	16	3.1	10.5	139	84	258	44	4.0	5.7	36	29	0.2
300 PPM	MEAN	0.05	16.3	3.12	10.85	150.3	92.0	169.5	51.0	4.32	6.10	34.3	32.3	0.10
	SE	0.03	0.6	0.07	0.26	11.7	2.7	31.9	9.3	0.11	0.15	1.8	3.3	0.06
	N	4	4	4	4	4	4	4	4	4	4	4	4	4
300 PPM	404	0.1	12	3.4	10.9	168	99	149	47	4.1	6.5	43	45	0.0
	409	0.0	11	3.0	10.5	116	93	86	34	4.1	5.8	30	26	0.0
	412	0.1	15	3.2	10.9	165	92	123	50	3.8	5.9	23	39	0.4
	414	0.1	12	3.4	10.9	152	104	88	72	4.6	6.9	26	19	0.2
300 PPM	MEAN	0.07	12.5	3.25	10.80	150.3	97.0	111.5	50.8	4.15	6.17	30.5	32.3	0.15
	SE	0.02	0.9	0.10	0.10	11.9	2.8	15.1	7.9	0.17	0.26	4.4	5.9	0.10
	N	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES--MALES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
1000 PPM	413	0.1	11	3.1	10.2	130	91	138	110	3.5	5.5	36	29	0.0
	416	0.1	11	3.2	10.7	93	88	170	28	4.1	5.4	35	41	0.0
	419	0.1	15	3.1	10.6	123	86	132	49	4.5	6.6	33	36	0.0
	420	0.1	11	2.9	10.3	113	84	150	43	4.4	6.0	42	72	0.1
MEAN SE N	0.10	0.10	12.0	3.07	10.45	114.8	87.3	147.5	57.5	4.12	5.87	36.5	44.5	0.02
	0.00	0.00	1.0	0.06	0.12	8.0	1.5	8.4	18.0	0.23	0.28	1.9	9.5	0.02
	4	4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-FEMALES
INTERVAL OF STUDY= WEEK 8

DOSE GROUP	ANIMAL NO	RILT	RUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
CONTROL	425	0.1	17	3.2	10.8	116	81	92	54	3.8	5.7	34	23	0.1
	426	0.1	12	3.4	11.1	144	99	161	62	3.5	6.8	38	39	0.3
	434	0.1	16	3.0	10.6	111	105	206	27	4.1	6.1	32	27	0.1
	435	0.1	13	3.2	10.4	129	75	220	29	4.0	5.8	36	38	0.0
	MEAN SF N	0.10 0.00 4	14.5 1.2 4	3.20 0.08 4	10.72 0.15 4	125.0 7.4 4	90.0 7.1 4	169.8 28.8 4	43.0 8.8 4	3.85 0.13 4	6.10 0.25 4	35.0 1.3 4	31.8 4.0 4	0.12 0.06 4
100 PPM	422	0.1	13	3.0	10.7	147	80	131	58	4.5	5.7	23	31	0.2
	429	0.1	15	3.0	10.9	165	76	173	78	3.9	6.1	39	28	1.2
	430	0.1	17	2.9	10.7	99	75	204	37	4.3	5.5	37	35	0.1
	431	0.1	16	3.4	10.6	106	86	133	45	4.0	5.8	26	27	0.2
	MEAN SL N	0.10 0.00 4	15.3 0.9 4	3.07 0.11 4	10.72 0.06 4	129.3 15.9 4	79.3 2.5 4	160.3 17.5 4	54.5 8.9 4	4.17 0.14 4	5.77 0.13 4	31.3 4.0 4	30.3 1.8 4	0.42 0.26 4
300 PPM	421	0.0	23	3.5	11.0	200	93	137	58	3.8	6.6	38	38	0.0
	424	0.1	12	3.3	10.8	145	88	153	63	4.2	6.3	31	37	0.2
	427	0.1 R	14 R	4.4 R	10.8	189 R	99 S	328 R	68 R	3.7 R	6.5 R	42 R	29 R	0.4 R
	433	0.0	18	3.0	10.8	220	93	129	76	4.7	6.4	32	27	0.5
	MEAN SC N	0.05 0.03 4	16.8 2.4 4	3.55 0.30 4	10.85 0.05 4	188.5 15.9 4	93.3 2.3 4	186.8 47.3 4	66.3 3 4	4.10 0.73 4	6.45 0.06 4	35.8 2.6 4	32.8 2.8 4	0.27 0.11 4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-FEMALES
INTERVAL OF STUDY= WEEK 9

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
1000 PPM	423	0.1	23	3.0	11.1	248	93	237	70	4.1	6.4	40	25	0.5
	432	0.1	13	2.9	9.7	102	80	263	62	4.6	5.5	41	30	0.1
	436	0.2	14	3.2	10.2	137	84	123	54	3.8	5.7	28	22	0.0
	437	0.1	17	3.5	11.4	180	72	126	61	4.3	6.0	32	36	0.4
MEAN SE N		0.12	16.8	3.15	10.60	166.8	82.3	187.3	61.8	4.20	5.90	35.3	28.3	0.25
		0.02	2.3	0.13	0.39	31.4	4.4	36.6	3.3	0.17	0.20	3.1	3.1	0.12
		4	4	4	4	4	4	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-MALES
INTERVAL OF STUDY= TERMINAL KILL

DOSE GROUP	ANIMAL NO	BILT	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
CONTROL	408	0.1	12	4.1	10.8	107	A	125	67	4.1	6.6	30	56	0.6
	411	0.1	20	3.9	11.9	103	77	243	43	3.5	6.8	31	24	0.5
	417	0.1	20	3.2	10.2	81	71	411	45	3.2	5.3	41	42	0.4
	418	0.1	13	4.2	11.0	108	A	175	65	3.8	6.4	24	46	0.6
	MEAN	0.10	16.3	3.85	10.97	98.5	74.0	238.5	55.0	3.75	6.27	31.5	42.0	0.52
	SE	0.00	2.2	0.23	0.35	6.0	3.0	62.4	6.4	0.13	0.34	3.5	6.7	0.05
	N	4	4	4	4	4	2	4	4	4	4	4	4	4
100 PPM	405	0.1	18	4.0	10.7	105	A	259	63	3.7	6.5	29	39	0.4
	406	0.1	16	4.3	11.2	122	A	243	35	3.6	6.5	29	30	0.5
	407	0.1	19	3.5	10.6	125	74	215	42	4.4	6.0	31	31	0.6
	415	0.1	14	3.4	10.1	104	74	337	45	3.2	5.4	36	37	0.4
	MEAN	0.10	16.8	3.80	10.65	114.0	74.0	263.5	46.3	3.72	6.10	31.3	34.3	0.47
	SE	0.00	1.1	0.21	0.23	5.5	0.0	26.1	6.0	0.25	0.26	1.7	2.2	0.05
	N	4	4	4	4	4	2	4	4	4	4	4	4	4
300 PPM	404	0.1	18	3.6	10.6	116	86	161	51	3.9	6.0	31	37	0.5
	409	0.1	19	3.3	10.8	96	79	230	36	4.0	6.5	29	31	0.5
	412	0.1	17	4.5	11.1	102	A	263	41	2.9	6.2	24	41	1.1
	414	0.1	12	4.3	10.8	109	A	113	53	3.2	6.8	24	23	1.1
	MEAN	0.10	16.5	3.92	10.82	105.8	82.5	191.8	45.3	3.50	6.37	27.0	33.0	0.80
	SE	0.00	1.6	0.28	0.10	4.3	3.5	33.8	4.0	0.27	0.18	1.8	3.9	0.17
	N	4	4	4	4	4	2	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES--MALES
INTERVAL OF STUDY= TERMINAL KILL

DOSE GROUP	ANIMAL NO	BILI	RUN	ALR	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
1000 PPM	413	0.1	16	2.9	10.0	105	88	268	81	3.0	5.3	30	29	0.9
	416	0.1	17	3.7	10.8	75	87	184	40	3.0	5.7	29	43	0.5
	419	0.1	14	4.1	11.0	90	- A	226	50	4.0	6.4	39	62	0.9
	420	0.1	15	4.3	10.9	101	- A	238	55	3.5	6.3	41	63	1.0
MEAN SF N		0.10	15.5	3.75	10.67	92.8	87.5*	229.0	56.5	3.38	5.92	34.8	49.3	0.82
		0.00	0.6	0.31	0.23	6.7	0.5	17.4	8.7	0.24	0.26	3.1	8.2	0.11
							2							

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-FEMALES
INTERVAL OF STUDY= TERMINAL KILL

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	PHOS	PROT	SGOT	SGPT	URIC
CONTROL	425	0.1	15	4.3	10.8	102	A	322	55	3.2	5.9	34	27	0.5
	426	0.2	13	4.2	11.0	110	A	239	49	3.2	6.4	29	37	0.6
	434	0.1	19	3.4	10.9	78	75	225	28	3.7	6.0	34	45	0.5
	435	0.1	16	3.4	10.8	105	81	211	31	3.1	5.8	32	31	0.5
	MEAN	0.12	15.8	3.82	10.87	98.8	78.0	249.3	40.8	3.30	6.02	32.3	35.0	0.52
	SE	0.02	1.3	0.25	0.05	7.1	3.0	24.9	6.6	0.14	0.13	1.2	3.9	0.03
	N	4	4	4	4	4	2	4	4	4	4	4	4	4
100 PPM	422	0.1	18	3.3	11.0	129	70	241	49	3.7	5.4	27	28	0.5
	429	0.1	14	3.9	10.4	154	A	240	80	2.9	5.9	32	33	0.4
	430	0.1	16	3.4	10.9	82	79	434	26	3.7	8.7	44	40	0.3
	431	0.1	19	4.3	10.7	96	A	175	33	3.4	5.7	21	35	0.5
	MEAN	0.10	16.8	3.72	10.75	115.3	74.5	272.5	47.0	3.42	6.42	31.0	34.0	0.42
	SE	0.00	1.1	0.23	0.13	16.2	4.5	56.0	12.0	0.19	0.77	4.9	2.5	0.05
	N	4	4	4	4	4	2	4	4	4	4	4	4	4
300 PPM	421	0.1	25	3.4	10.8	157	80	364	45	3.3	6.4	38	35	0.7
	424	0.1	12	4.1	10.5	114	A	200	61	2.9	5.7	21	32	1.2
	427	0.1	15	4.0	10.9	159	A	236	49	3.3	5.8	17	25	0.9
	433	0.1	20	3.4	11.1	132	73	315	66	5.0	5.8	35	39	0.5
	MEAN	0.10	18.0	3.72	10.82	140.5	76.5	278.8	55.3	3.62	5.92	27.8	32.8	0.82
	SE	0.00	2.9	0.19	0.13	10.8	3.5	37.2	4.9	0.47	0.16	4.2	3.0	0.15
	N	4	4	4	4	4	2	4	4	4	4	4	4	4

TABLE II-F-48 (Continued)

CLINICAL CHEMISTRIES FOR P-1073409

TABLE 5 (CONTINUED)
INDIVIDUAL ANIMAL CHEMISTRIES-F1MALFS
INTERVAL OF STUDY= TERMINAL KILL

DOSE GROUP	ANIMAL NO	BILI	BUN	ALB	CA	CHOL	GLCS	LDH	SAP	CHOS	PROT	SGOT	SGPT	URIC
1000 PPH	423	0.2	24	3.4	11.4	120	81	340	48	3.8	6.2	39	29	0.6
	432	0.1	15	4.0	10.2	86	A	241	59	3.8	5.5	29	32	1.1
	436	0.1	16	4.1	10.3	138	A	171	54	3.2	5.6	19	19	0.8
	437	0.1	15	3.7	11.5	136	69	274	55	3.6	6.0	32	38	0.3
MEAN SE N		0.12	17.5	3.80	10.85	120.0	74.5	256.5	54.0	3.60	5.82	29.8	29.5	0.70
		0.02	2.2	0.16	0.35	12.0	6.5	35.2	2.3	0.14	0.17	4.2	4.0	0.17
		4	4	4	4	4	2	4	4	4	4	4	4	4

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6

KEY TO URINALYSIS

Color:	Y = Yellow Str = Straw Amb = Amber Br = Brown Or = Orange Ply = Pale Yellow PlOr = Pale Orange None = Colorless
Appearance:	Cldy = Cloudy Sl Cldy = Slightly Cloudy
Albumin:	0 = Negative + = Positive ± = Trace 1+ = 30 mg 2+ = 100 mg 3+ = 300 mg 4+ = 100 mg or greater
Other:	- or 0 = None seen or Negative ± = Trace, Rare, Occasional 1+ = Slight, Small, Little 2+ = Moderate, Frequent 3+ = Severe, Heavy, Large, Many 4+ = Maximal
Microscopic Examination:	HPF = High power field
Crystals:	UA = Uric Acid TP = Triple Phosphate CaOx = Calcium Oxalate
Other:	Yst = Yeast Mu = Mucous Threads Sp = Sperm HA = Hippuric Acid

TABLE II-F-49

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6

URINALYSIS

INITIAL

MALES

DOSE LEVEL (PPH)	ANIMAL NUMBER	COLOR	APPEAR- ANCE	SPEC. GRAV.	pH	AL- BUMIN	GLU- COSE	KE- TONES	BILI- RUBIN	OCCULT BLOOD	WBC	RBC	CASTS	EPITH	AMORPH	BACT	CRYSTALS	OTHER	MUCOUS THREADS
0	408	Y	Hazy	1.017	7	0	0	0	±	0	0	-	-	2+	3+	3+	TP 2+	-	-
	411	Y	Hazy	1.040	7	0	0	0	±	0	0-5	-	-	1+	-	3+	-	Ha ±, Sp 2+	-
	417	Amb	Cl dy	1.045	7	1+	0	0	1+	2+	-	-	2+	2+	2+	2+	TP 2+	-	-
	418	Amb	Hazy	1.040	9	3+	0	0	2+	0	0-1	-	-	1+	1+	4+	UA 2+, TP 2+	Ha ±, Sp 1+	-
100	405	Amb	Cl dy	>1.045	8	3+	0	0	3+	0	-	-	-	1+	1+	±	TP 3+	-	-
	406	Y	Cl dy	1.037	8	0	0	0	1+	0	-	-	-	1+	1+	3+	TP 1+	Ha 1+	-
	407	Y	Cl dy	1.030	8	0	0	0	±	0	-	-	-	2+	2+	4+	-	-	-
	415	Str	Hazy	1.007	7	0	0	0	0	0	-	-	-	-	2+	±	-	-	-
300	404	Amb	Hazy	1.045	8	1+	0	0	1+	0	0-1	-	-	1+	1+	3+	TP 3+	Ha ±	-
	409	Y	Cl dy	1.035	8	1+	0	0	±	0	0-1	0-1	-	1+	2+	4+	TP 2+	Ha 1+	-
	412	Amb	Turbid	>1.045	9	±	0	0	0	0	±	±	0	2+	±	2+	TP 3+	-	-
	414	Amb	Hazy	1.040	6	1+	0	0	0	0	-	-	-	±	1+	2+	UA ±, TP ±	-	-
1000	413	Amb	Cl dy	>1.045	7	2+	0	0	2+	0	0-2	0-1	-	1+	1+	1+	TP 3+	Ha 1+, Sp 1+	-
	416	Y	Cl dy	>1.045	9	1+	0	0	±	0	-	-	-	±	1+	4+	UA 1+, TP 2+	-	-
	419	Y	Hazy	1.030	6	0	0	0	1+	0	-	-	-	3+	±	±	UA ±	-	-
	420	Y	Cl dy	1.010	6	±	0	0	0	0	-	-	-	2+	2+	3+	UA ±, TP 1+	-	-

TABLE II-F-49 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6 (CONTINUED)

URINALYSIS

INITIAL

FEMALES

DOSE LEVEL (PPH)	ANIMAL NUMBER	COLOR	APPEAR- ANCE	SPEC. GRAV.	pH	AL- BUMIN	GLU- COSE	KE- TONES	BILI- RUBIN	OCCULT BLOOD	WBC	EST. CASTS	EPITH	AMORPH	BACT CRYSTALS	OTHER	MUCOUS THREADS
0	425	Amb	Cl dy	1.035	7	1+	0	0	0	0	-	-	±	2+	3+	TP 3+	-
	426	Amb	Hazy	1.030	8	0	0	0	0	0	2-8	-	3+	1+	1+ UA 1+, TP 1+	Ha 1+	-
	434	Y	Hazy	1.017	8	0	0	0	0	0	0-1	-	1+	2+	4+ TP 3+	Ha 1+	-
	435	Amb	Cl dy	>1.045	7	0	0	0	0	0	0-1	0-1	2+	2+	2+ UA ±, TP 4+	-	-
100	422	Y	Hazy	>1.045	7	±	0	0	0	0	0-1	-	2+	2+	± TP 2+	Ha 2+	-
	429	Y	Cl dy	1.010	8	±	0	0	0	0	-	-	±	1+	4+ TP ±	Ha 2+	-
	430	Y	Cl dy	>1.045	6	0	0	0	0	0	-	-	1+	1+	1+ TP ±	Ha 1+	-
	431	Amb	Cl dy	1.025	7	±	0	0	0	0	-	-	-	1+	3+ TP 1+	-	-
300	421	Y	Hazy	1.040	6	0	0	0	0	0	0-1	0-1	3+	±	±	-	-
	424	Y	Cl dy	1.045	7	0	0	0	0	0	-	0-2	±	2+	1+ TP 2+	-	-
	427	Amb	Hazy	>1.045	6	1+	0	0	0	0	0-1	-	2+	1+	1+ UA 1+, TP 2+	Ha 1+	-
	433	Amb	Cl dy	1.035	6	1+	0	0	3+	2+	-	-	1+	3+	2+ UA ±	-	-
1000	423	Y	Hazy	1.018	7	±	0	0	0	±	-	-	±	2+	4+ UA 1+, TP 1+	-	-
	432	Br	Cl dy	1.035	7	±	0	0	0	1+	0-1	-	1+	2+	4+ TP 1+	-	-
	436	Amb	Hazy	1.044	8	1+	0	0	0	0	0-2	0-4	2+	1+	1+ TP 2+	-	-
	437	Y	Cl dy	1.030	9	±	0	0	0	0	-	-	1+	1+	2+ TP 1+	Ha 2+	-

TABLE II-F-49 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6 (CONTINUED)

URINALYSIS

WEEK 8

MALES

DOSE LEVEL (PPM)	ANIMAL NUMBER	COLOR	APPEAR- ANCE	SPEC. GRAV.	pH	AL- BUMIN	GLU- COSE	KE- TONES	BILI- RUBIN	OCCULT BLOOD	WBC	RBC	CASTS	EPITH	AMORPH	BACT CRYSTALS	OTHER	MUCOUS THREADS
0	425	Y	Clidy	1.024	9	2+	0	0	1+	0	-	-	-	-	-	4+ TP 2+	-	-
	426	Y	Clidy	1.027	9	1+	0	0	2+	0	-	-	-	-	-	4+ TP 4+	-	-
	434	Str	Hazy	1.045	9	0	0	0	0	0	-	-	-	-	1+	2+ TP 1	-	-
	435	Y	Clidy	1.027	6	0	0	0	0	0	-	-	-	-	±	1+ TP 1	-	-
100	405	Y	Clidy	1.030	9	1+	0	0	2+	0	3-5	-	-	-	-	4+ TP 4+	-	-
	406	Y	Clidy	1.016	9	1+	0	0	2+	0	0	-	-	-	-	4+ TP 4+	-	-
	407	Str	Turbid	1.050	9	0	0	0	0	0	-	-	-	-	1+	3+ TP 1	Sp +	-
	415	Br	Turbid	1.062	9	0	0	0	0	0	0	-	-	-	-	2+	-	-
300	404	Y	Clidy	1.022	7	0	0	0	0	0	2-3	-	-	6-8	-	4+ TP 4+	-	-
	409	Y	Clidy	1.058	8	1+	0	0	1+	0	3-5	-	-	-	2+	2+ TP 4+	-	-
	412	Y	Hazy	1.010	9	0	0	0	0	0	0	-	-	-	±	3+ TP 1+	-	-
	414	Y	Hazy	1.014	8	0	0	0	0	0	0	-	-	-	±	2+ TP 1+	-	-
1000	413	Y	Clidy	1.032	9	1+	0	0	2+	0	0	-	-	-	-	4+ TP 4+	-	-
	416	f	Clidy	1.030	9	0	0	0	0	0	0	-	-	-	-	4+ TP 4+	-	-
	419	Str	Turbid	1.050	6	0	0	0	0	0	5-10	-	-	1-2	1+	±	Sp ±	-
	420	Y	Hazy	1.026	7	±	0	0	0	0	0	-	-	5-10	1+	1+ TP 1+	Sp ±	-

TABLE II-F-49 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6 (CONTINUED)

URINALYSIS

WEEK 8

FEMALES

DOSE LEVEL (PPH)	ANIMAL NUMBER	COLOR	APPEAR- ANCE	SPEC. GRAV.	pH	AL BUMIN	GLU- COSE	KE- TONES	BILI- RUBIN	OCCULT BLOOD	HBC	RBC	CASTS	EPITH	AMORPH	BACI	CRYSTALS	OTIHP	MUCOUS THREADS
0	408	Y	Cldy	1.010	9	2+	0	0	1+	0	-	-	-	-	-	4+	TP 1+	-	-
	411	Str	Hazy	1.057	6	0	0	0	0	0	-	-	-	-	1+	-	TP 3+	Sp 1+	-
	417	Y	Turbid	1.045	6	±	0	0	0	2+	-	-	-	-	3+	1+	TP 1	Sp	-
100	418	Y	Cldy	1.042	9	±	0	0	4+	0	3-5	-	-	-	-	4+	TP 2+	-	-
	422	Str	Hazy	1.047	6	0	0	0	0	0	6-12	-	-	6-8	±	-	TP 4+	-	-
	429	Y	Cldy	1.029	8	1+	0	0	0	0	-	-	-	-	3+	4+	TP 3+	-	-
300	430	Br	Hazy	1.062	8	0	0	0	0	0	-	-	-	-	-	-	TP 4+	-	-
	431	Y	Cldy	1.034	9	2+	0	0	4+	0	-	-	-	-	-	4+	TP 4+	-	-
	421	Y	Cldy	1.047	9	1+	0	0	4+	0	-	-	-	-	-	4+	TP 4+	-	-
1000	424	Str	Hazy	1.055	9	0	0	0	0	0	0-1	-	-	0-1	1+	-	TP 1+	-	-
	427	Str	Hazy	1.037	8	0	0	0	0	0	-	-	-	-	1+	2+	TP 2+	-	-
	433	Y	Cldy	1.031	9	1+	0	0	3+	0	-	-	-	-	-	4+	TP 4+	-	-
1000	425	Y	Hazy	1.021	9	1+	0	0	2+	0	-	-	-	-	-	4+	TP 1+	-	-
	432	Str	Hazy	1.025	9	0	0	0	0	0	-	-	-	-	2+	2+	TP ±	-	-
	416	Str	Hazy	1.040	7	0	0	0	0	0	-	-	-	3-6	+	-	TP 4+	-	-
	437	Y	Cldy	1.023	9	3+	0	0	3+	0	-	-	-	-	-	-	TP 4+	-	-

TABLE II-F-49 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6 (CONTINUED)

URINALYSIS

WEEK 13

MALES

DOSE LEVEL (PPM)	ANIMAL NUMBER	COLOR	APPEAR- ANCE	SPEC. GRAV.	pH	AL- BUMIN	GLU- COSE	KE- TONES	BILI- RUBIN	OCCULT BLOOD	RBC	CASTS	EPITH	AMORPH	BACT CRYSTALS	OTHER	THREEFOLD
0	425	Y	Hazy	1.024	9	0	0	0	0	0	-	-	-	3+	2+	TP 1+	-
	426	Y	Hazy	1.012	9	0	0	0	0	0	-	-	-	2+	2+	TP ±	-
	434	Y	Turbid	1.019	9	0	0	0	0	0	-	-	-	3+	-	TP ±	-
	435	Br	Turbid	1.038	8	0	0	0	0	0	-	-	-	2+	-	TP 3+	-
100	405	Y	Hazy	1.019	8	0	0	0	0	0	0-1	-	-	1+	2+	TP ±	-
	406	Y	Hazy	1.018	8	0	0	0	0	0	-	-	-	2+	2+	TP ±	-
	407	Y	Turbid	1.023	6	0	0	0	0	0	-	-	-	3+	-	-	-
	415	Y	Turbid	1.019	9	0	0	0	0	0	-	-	-	3+	-	TP 1+	-
300	404	Y	Turbid	1.028	9	0	0	0	0	0	-	-	-	3+	2+	TP ±	-
	409	Str	Turbid	1.053	9	0	0	0	0	0	-	-	-	3+	2+	TP 1+	-
	412	Str	Turbid	1.058	9	0	0	0	0	0	-	-	-	3+	-	TP ±	-
	414	Str	Turbid	1.023	9	0	0	0	0	0	-	-	-	3+	-	TP 1+	-
1000	413	Str	Hazy	1.030	8	0	0	0	0	0	0-3	-	-	2+	-	TP 1+	Sp 2+
	416	Str	Hazy	1.032	7	0	0	0	0	0	-	-	-	2+	-	TP 1+	Sp 2+
	419	Str	Turbid	1.040	7	0	0	0	0	0	-	-	-	3+	3+	TP 2+	-
	420	Y	Turbid	1.027	9	0	0	0	0	0	-	-	-	3+	-	TP 1+	-

TABLE II-F-49 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 6 (CONTINUED)

URINALYSIS

WEEK 13

FEMALES

DOSE LEVEL (PPM)	ANIMAL NUMBER	COLOR	APPEAR- ANCE	SPEC. GRAV	pH	AL- BUMIN	GLU- COSE	KE- TONES	BILI- RUBIN	OCCULT BLOOD	RBC	CASTS	EPITH	AMORPH	BACT CRYST	OTHER	MUCOUS THREADS
0	408	Str	Hazy	1.011	9	0	0	0	0	0	-	-	-	2+	TP 1+	-	-
	411	Str	Hazy	1.041	8	0	0	0	0	0	-	-	-	2+	TP 1+	-	-
	417	Str	Turbid	1.037	8	±	0	0	0	0	-	-	-	3+	TP 2+	-	-
	418	Y	Hazy	1.008	9	0	0	0	0	0	-	-	-	2+	TP 1+	-	-
100	422	Y	Turbid	1.026	9	±	0	0	0	0	-	-	-	3+	TP 1+	-	-
	429	Str	Turbid	1.055	9	0	0	0	0	0	0-1	-	-	2+	TP 1+	-	1+
	430	Y	Turbid	1.032	8	0	0	0	0	0	-	-	-	3+	TP 2+	-	-
	431	Y	Turbid	1.034	9	0	0	0	0	0	0-3	-	0-6	3+	TP 2+	-	±
300	421	Br	Turbid	1.027	9	±	0	0	0	±	-	-	-	3+	TP 2+	-	-
	424	Y	Turbid	1.022	9	0	0	0	0	0	-	-	-	3+	TP 1+	-	-
	427	Br	Turbid	1.098	9	0	0	0	0	0	-	-	-	2+	TP 1+	-	2+
	433	Str	Turbid	1.048	9	0	0	0	0	0	-	-	-	3+	-	-	-
1000	423	Y	Clay	1.016	9	±	0	0	0	0	-	-	-	2+	TP 1+	-	-
	432	Str	Hazy	1.034	8	0	0	0	0	0	0-3	-	-	2+	TP 1+	-	2+
	436	Y	Hazy	1.004	9	0	0	0	0	0	-	-	-	3+	TP 1+	-	-
	437	Y	Hazy	1.016	9	0	0	0	0	0	-	-	-	1+	TP 1+	-	-

TABLE II-F-50

TABLE 7

PROJECT NO. 1073409
 ORGAN WEIGHTS IN MALE DOGS (GRAMS)
 DOSE - 0 PPM
 GROUP - 1
 TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0402	15800.0	77.2990	0.0 ^a	112.2280	443.8350	74.6140	81.7960	1.1300	23.1770
0411	9800.0	80.9100	1.0340	71.3120	301.4540	58.4590	49.0490	0.7170	15.8160
0417	9200.0	76.6100	0.6700	85.3400	207.0000	73.5400	57.5000	0.8200	19.3100
0418	8700.0	79.4800	0.9200	77.9500	270.7800	104.3000	58.8500	1.9000	22.7600
N	4	4	3	4	4	4	4	4	4
MEAN	10875.0	78.5747	0.8747	80.8325	322.0172	77.7282	61.7987	1.1417	20.2657
S.D.	3314.0	1.9800	0.1862	17.8108	85.9413	19.1886	14.0193	0.5351	3.4356
S.E.	1657.0	0.9900	0.1075	8.9054	42.9707	9.5943	7.0096	0.2676	1.7178

^aLeft thyroid not located.

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)

PROJECT NO. 1073409

ORGAN WEIGHTS 1% MALE DOGS (GRAMS)

DOSE - 100 PPM

GROUP - 2

TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0405	12000.0	79.2890	0.6820	87.8710	327.2300	87.3910	61.4320	0.9310	19.2260
0406	12400.0	100.3700	0.6900	91.4000	289.9700	100.7200	63.4200	1.1700	20.4700
0407	10900.0	86.6800	0.3100	91.9100	335.9300	66.1100	67.2000	1.3900	24.1400
0415	11200.0	89.8100	0.9100	81.1400	258.5300	127.7700	49.0900	0.8100	19.9800
N	4	4	4	4	4	4	4	4	4
MEAN	11625.0	89.0372*	0.7730	85.0802	302.9150	95.4977	60.2855	1.0752	20.9540
S.D.	694.6	8.7486	0.1085	4.9632	35.6782	25.8078	7.8377	0.2577	2.1848
S.E.	347.3	4.3743	0.0542	2.4816	17.8391	12.9039	3.9189	0.1268	1.0924

* $p < 0.05$ as compared to controls: Dunnett's t-test.

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)

PROJECT NO. 1073409
 ORGAN WEIGHTS IN MALE DOGS (GRAMS)
 DOSE - 300 PPM
 GROUP - 3
 TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0404	10800.0	79.9230	0.8040	90.3000	318.4690	92.5170	49.3080	0.8760	18.3230
0409	9400.0	82.2420	0.3350	76.7530	250.7610	87.2330	57.9380	0.4100	15.7750
0412	8700.0	84.1800	0.5000	68.9300	233.9900	96.8500	45.3700	1.8500	17.0000
0414	10400.0	93.2900	0.8400	93.4000	285.1100	81.6300	62.3300	1.3500	25.4800
N	4	4	4	4	4	4	4	4	4
MEAN	9825.0	84.9087	0.6197	82.3457	272.0625	89.5575	53.7365	1.1215	19.1445
S.D.	953.5	5.8522	0.2435	11.4997	37.5309	6.5876	7.7598	0.6190	4.3499
S.E.	476.8	2.9261	0.1218	5.7498	18.7685	3.2938	3.8849	0.3095	2.1750

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)

PROJECT NO. 1073439
 ORGAN WEIGHTS IN MALE DOGS (GRAMS)
 DOSE - 1000 PPV
 GROUP - 4
 TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0419	10600.0	77.5800	0.8270	74.0900	270.6200	85.5100	48.6000	1.0400	14.3700
0416	9400.0	79.3500	0.4100	75.6700	305.8500	87.4800	56.6200	1.6500	22.3000
0413	10700.0	77.4270	0.7710	95.9420	292.5060	132.3990	63.3780	0.9330	21.6440
0420	10900.0	82.5300	0.5900	78.5600	274.8400	84.6500	55.0200	0.7400	16.4300
N	4	4	4	4	4	4	4	4	4
MEAN	10400.0	79.2218	0.6477	81.0655	285.9540	97.5097	55.9045	1.0907	18.6860
S.D.	678.2	2.3719	0.1868	10.0689	16.3037	23.2396	6.0691	0.3930	3.8956
S.E.	339.1	1.1859	0.0934	5.0444	8.1518	11.6448	3.0346	0.1965	1.9478

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)

PROJECT NO. 1073409
 ORGAN WEIGHTS IN FEMALE GLUS (GRAMS)
 DOSE - 0 PPM
 GROUP - 1
 TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0425	8300.0	76.0000	0.5500	65.0220	229.6410	96.0820	41.0390	0.9580	0.1490
0426	7600.0	64.0300	0.5800	65.2500	224.8400	88.1400	44.7900	0.9800	0.5300
0434	7900.0	73.1700	0.5300	71.3600	210.8000	86.1300	42.0700	0.6400	0.5500
0435	6400.0	68.6700	0.3100	68.3700	203.3700	52.5300	28.3400	0.9200	0.5600
N	4	4	4	4	4	4	4	4	4
MEAN	7550.0	75.4695	0.4940	67.5255	217.1627	80.7205	39.0597	0.8745	0.4472
S.D.	816.5	6.4574	0.1244	3.2097	12.1843	19.2786	7.3196	0.1583	0.1992
S.E.	409.3	3.2287	0.0522	1.6048	6.0921	9.6393	3.6598	0.0791	0.0996

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)

PROJECT NO. 1073409

ORGAN WEIGHTS IN FEMALE DOGS (GRAMS)

DOSE - 100 PPM

GROUP - 2

TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0422	8700.0	70.9690	0.5450	68.4280	227.3220	63.3320	35.0820	0.8030	1.1070
0429	9600.0	84.7690	0.6980	75.1000	303.8470	90.0000	42.2570	0.8880	1.3080
0430	9100.0	79.7000	1.0000	69.3300	241.8500	81.7900	47.5600	1.2100	2.0400
0431	7800.0	81.9400	0.5400	62.7100	250.0700	71.1300	39.0900	0.8500	1.3000
N	4	4	4	4	4	4	4	4	4
MEAN	8800.0	79.3445	0.6957	68.8920	255.7722	76.5630	40.9972	0.7627	1.4387
S.D.	761.6	5.9564	0.2157	5.0716	33.4013	11.7253	5.2689	0.4241	0.4115
S.E.	380.8	2.9782	0.1078	2.5358	16.7007	5.8627	2.6345	0.2121	0.2057

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)

PROJECT NO. 1077404
 100% AROMATIS 10 FEMALE DOGS (GRAMS)
 DOSE - 100 PPM
 GROUP 1
 TREATMENT WILL

ANIMAL NO.	BODY WEIGHT	SPAD	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0421	1000.0	77.0000	0.7500	72.5000	307.5570	32.4000	43.2070	0.9030	3.0510
0422	7500.0	74.0100	0.7200	71.3000	249.4200	110.3400	43.4400	0.7000	0.0100
0427	7300.0	70.7000	0.6300	54.0000	223.1300	50.9000	39.5000	0.6900	0.9300
0428	9400.0	84.4100	0.4700	60.1200	229.3300	93.3000	39.2100	0.6700	0.7500
		4	4	4	4	4	4	4	4
0429	5850.0	70.2700	0.6425	64.5387	252.5592	31.7350	42.5392	0.8057	1.4852
0430	10340.0	4.7000	0.1200	3.9097	38.4713	13.5750	4.2027	0.0964	1.4496
0431	917.0	2.1200	0.0625	4.4840	19.2357	6.7693	2.1310	0.0492	0.7249

TABLE II-F-50 (Continued)

TABLE 7 (CONTINUED)
 PROJECT NO. 1073409
 ORGAN WEIGHTS IN FEMALE DOGS (GRAMS)
 DOSE - 1000 PPM
 GROUP - 4
 TERMINAL KILL

ANIMAL NUMBER	BODY WEIGHT	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0432	7400.0	75.6790	0.9490	81.2840	213.0240	136.4550	46.2180	1.0210	0.5970
0436	7900.0	0.0 ^b	0.5000	53.6200	199.5000	77.0900	39.2500	0.9800	1.0200
0437	8800.0	72.4700	0.5700	72.9500	254.5200	95.7400	41.0700	0.7800	0.5300
0423	7600.0	69.2230	0.4360	56.0610	206.0600	57.4130	39.1030	0.3170	0.9450
N	4	3	4	4	4	4	4	4	4
MEAN	7925.0	72.4573	0.6137	65.9787	218.2760	91.6745	41.4102	0.7745	0.7730
S.D.	618.5	3.2280	0.2301	13.3410	24.7856	33.7065	3.3277	0.3227	0.2454
S.E.	309.2	1.8637	0.1151	6.6705	12.3928	16.8532	1.6638	0.1613	0.1227

^bWeight not taken.

TABLE II-F-51

TABLE 8

PROJECT NO. 1073409
 ORGAN WEIGHT-BODY WEIGHT PERCENTAGES IN MALE DOGS
 DOSE - 0 PPM
 GROUP - 1
 TERMINAL KILL

ANIMAL NUMBER	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0408	0.4892	0.0 ^a	0.7103	2.8407	0.4722	0.5177	0.0072	0.1467
0411	0.8256	0.0106	0.7328	3.0761	0.5965	0.5005	0.0073	0.1614
0417	0.8327	0.0073	0.9276	2.9022	0.7993	0.6250	0.0089	0.2099
0419	0.9136	0.0106	0.8960	3.1124	1.1988	0.6764	0.0218	0.2616
N	4	3	4	4	4	4	4	4
MEAN	0.7653	0.0095	0.8167	2.9828	0.7667	0.5799	0.0113	0.1949
S.D.	0.1883	0.0019	0.1110	0.1319	0.3181	0.0847	0.0071	0.0520
S.E.	0.0942	0.0011	0.0555	0.0659	0.1590	0.0424	0.0035	0.0260

^aLeft thyroid not located.

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

PROJECT NO. 1073400
 ORGAN WEIGHT-BODY WEIGHT PERCENTAGES IN MALE J000
 DOSE - 100 PPV
 GROUP - 2
 TEST 1.01 KILL

ANIMAL NUMBER	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0405	0.0007	0.0057	0.7323	2.7209	0.7283	0.5119	0.0078	0.1602
0406	0.0094	0.0056	0.7371	2.3385	0.5123	0.5115	0.0094	0.1651
0407	0.7952	0.0074	0.8432	3.0819	0.5565	0.6105	0.0128	0.2215
0415	0.3919	0.0051	0.7245	2.3053	1.1408	0.4383	0.0072	0.1784
N	4	4	4	4	4	4	4	4
MEAN	0.7600	0.0067	0.7592	2.6135	0.8220	0.5196	0.0093	0.1813
S.D.	0.0710	0.0013	0.0567	0.3656	0.2257	0.0733	0.0025	0.0279
S.E.	0.0155	0.0006	0.0281	0.1825	0.1144	0.0367	0.0012	0.0139

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

PROJECT NO. 1073404

ORGAN WEIGHT-BODY WEIGHT PERCENTAGES IN MALL DOGS

WEIGHT = 300 POUNDS

SEX = M

TREATMENT = NONE

ANIMAL NUMBER	SPLEEN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	TESTES
0404	0.7400	0.0074	0.9361	2.7444	0.3566	0.4566	0.0081	0.1697
0405	0.5749	0.0036	0.8155	2.6677	0.5280	0.6164	0.0044	0.1678
0412	0.0670	0.0057	0.7923	2.6895	1.1132	0.5215	0.0213	0.1954
0414	0.8970	0.0081	0.9921	2.7414	0.7849	0.5993	0.0130	0.2450
	4	4	4	4	4	4	4	4
MEAN	0.5699	0.0062	0.8354	2.7014	0.9207	0.5434	0.0117	0.1945
S.D.	0.0652	0.0020	0.0452	0.1234	0.1410	0.0730	0.0073	0.0360
S.E.	0.0470	0.0010	0.0220	0.0642	0.0705	0.0369	0.0036	0.0180

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

PROJECT NO. 1072405
 TISSUE WEIGHT-BODY WEIGHT PERCENTAGES IN MALE DOGS
 DOSE - 1000 PPV
 GROUP - 4
 TERMINAL KILL

ANIMAL NUMBER	BRAIN	THYROID	HEART	LIVER	SPLLEN	KIDNEYS	ADRENALS	TESTES
0419	0.7310	0.0077	0.0990	0.5530	0.2067	0.4585	0.0095	0.1350
0416	0.6441	0.0044	0.0950	0.2537	0.4300	0.6023	0.0176	0.2372
0417	0.7230	0.0072	0.0967	0.7537	1.2374	0.5923	0.0007	0.2023
0420	0.7572	0.0054	0.7207	2.5215	0.7700	0.5046	0.0056	0.1507
	4	4	4	4	4	4	4	4
MEAN	0.7042	0.0062	0.7803	2.7055	0.9370	0.5395	0.0107	0.1815
S.D.	0.0552	0.0016	0.0900	0.3337	0.2105	0.0695	0.0047	0.0465
S.E.	0.0276	0.0008	0.0450	0.1695	0.1053	0.0348	0.0024	0.0234

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

24 JUL 1964

GROSS WEIGHT-BODY WEIGHT PERCENTAGES IN FEMALE DOGS

SEX - F

AGE - 1

TREATMENT

ANIMAL NUMBER	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0425	0.9153	0.0067	0.7434	2.7609	1.1576	0.4944	0.0115	0.0018
0426	1.1157	0.0076	0.8536	2.9544	1.1597	0.5893	0.0129	0.0070
0434	0.8262	0.0067	0.9390	2.4644	1.0903	0.5325	0.0081	0.0070
0435	1.0730	0.0048	1.0683	3.1777	0.8208	0.4426	0.0144	0.0087
N	4	4	4	4	4	4	4	4
MEAN	1.0251	0.0069	0.9052	2.9923	1.0571	0.5148	0.0117	0.0061
S.D.	0.0982	0.0012	0.1204	0.2249	0.1606	0.0618	0.0027	0.0030
S.E.	0.0491	0.0006	0.0602	0.1124	0.0804	0.0309	0.0013	0.0015

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

PROJECT NO. 1073409

ORGAN WEIGHT-BODY WEIGHT PERCENTAGES IN FEMALE DOGS

DOSE - 100 PPM

GROUP - 2

TERMINAL KILL

ANIMAL NUMBER	SKIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0422	0.8157	0.0063	0.7865	2.6129	0.7280	0.4032	0.0092	0.0127
0429	0.8830	0.0073	0.7823	3.1651	0.9375	0.4402	0.0020	0.0136
0430	0.8758	0.0110	0.7619	2.6577	0.8988	0.5226	0.0133	0.0224
0431	1.0505	0.0069	0.8040	3.2060	0.9119	0.5012	0.0109	0.0167
N	4	4	4	4	4	4	4	4
MEAN	0.9063	0.0079	0.7837	2.9104	0.8690	0.4668	0.0088	0.0164
S.D.	0.1008	0.0021	0.0173	0.3187	0.0954	0.0549	0.0049	0.0044
S.E.	0.0504	0.0011	0.0086	0.1593	0.0477	0.0275	0.0024	0.0022

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

U.S. DEPARTMENT OF AGRICULTURE
BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C. 20250
PLANT INDUSTRY
PLANT INDUSTRY

ANIMAL NUMBER	SPLEEN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
3421	0.7100	0.0069	0.6675	2.8710	0.7560	0.4423	0.0083	0.0335
3424	0.9500	0.0012	0.9149	3.1577	1.4140	0.5000	0.0047	0.0078
3427	1.0507	0.0060	0.7547	1.0500	1.1050	0.5384	0.0090	0.0127
3428	0.4681	0.0080	0.6370	2.4557	0.9420	0.4171	0.0093	0.0080
MEAN	0.9010	0.0074	0.7400	2.8709	1.0570	0.4887	0.0092	0.0155
S.D.	0.1437	0.0019	0.1241	0.3314	0.2730	0.0693	0.0000	0.0122
S.E.	0.0719	0.0010	0.0620	0.1657	0.1360	0.0340	0.0003	0.0061

TABLE II-F-51 (Continued)

TABLE 8 (CONTINUED)

PROJECT NO. 1073409
 ORGAN WEIGHT-BODY WEIGHT PERCENTAGES IN FEMALE DOGS
 DOSE - 1000 PPM
 GROUP - 4
 TERMINAL KILL

ANIMAL NUMBER	BRAIN	THYROID	HEART	LIVER	SPLEEN	KIDNEYS	ADRENALS	OVARIES
0432	1.0227	0.0128	1.0984	2.8787	1.8440	0.6246	0.0138	0.0081
0436	0.0 b	0.0063	0.6787	2.5253	0.9758	0.4968	0.0124	0.0129
0437	0.8235	0.0065	0.8290	2.8923	1.0880	0.4667	0.0089	0.0060
0423	0.9108	0.0057	0.7376	2.7113	0.7554	0.5145	0.0042	0.0124
N	3	4	4	4	4	4	4	4
MEAN	0.9190	0.0078	0.8359	2.7519	1.1658	0.5257	0.0098	0.0099
S.D.	0.0998	0.0033	0.1856	0.1720	0.4728	0.0688	0.0043	0.0034
S.E.	0.0576	0.0017	0.0928	0.0860	0.2364	0.0344	0.0021	0.0017

^bWeight not taken.

SPONSOR: U.S. Army Medical Bioengineering R&D Laboratory

MATERIAL: Dicyclopentadiene (DCPD)

SUBJECT: CHEMISTRY REPORT
Analysis of Diet Formulations
LBI Project No. 10734-09

1. OBJECTIVE

The objective of this study was to analyze DCPD in animal chow with regard to stability and formulation content in diet.

2. MATERIALS

This method describes the analytical procedure for the determination of DCPD in dosed feed used by LBI during the course of the study. A 5 g feed subsample is extracted with 20 ml of diethyl ether by shaking for 15 minutes in an automated shaker. The extract is clarified by centrifugation for 10 minutes at 1350 rpm. The extracts are analyzed with a Varian 2100 gas chromatograph equipped with flame ionization detectors. The DCPD content is calculated from a calibration curve obtained by GLC analysis of reference solutions of DCPD in ether. Control and spiked control feed samples are analyzed concurrently to correct for possible feed background and compound recovery.

The following equipment and supplies were used.

- a. Graduated conical Falcon tubes, 50 ml, with positive seal caps (available from Becton, Dickinson and Company, Oxnard, CA, 93030; stock number H8292-209811).
- b. Volumetric glassware - 1, 4, 5 and 10 ml pipettes; 50 and 100 ml flasks.
- c. Graduated cylinder - 25 ml capacity.
- d. Graduated glass centrifuge tubes, 15 ml, with ground glass stoppers.
- e. Mechanical shaker.
- f. Centrifuge.
- g. Analytical laboratory balance (accurate to 0.01 mg).
- h. Top-loading laboratory balance (accurate to 0.01 g).

2. MATERIALS (Continued)

- i. Gas-liquid chromatograph - Varian 2100, equipped with a 1.8 m x 2 mm I.D. glass column packed with 10% FFAP on 80/100 mesh Supelcoport, flame ionization detectors.
- j. Diethyl ether (Burdick and Jackson).
- k. Dicyclopentadiene.

3. EXPERIMENTAL DESIGN

A stock standard solution of DCPD is prepared by dissolving 50 mg of DCPD in 50 ml of acetone. Take a 5 ml aliquot and dilute to 100 ml with diethyl ether in a volumetric flask. This solution has a concentration of 0.05 mg/ml. Prepare a standard curve by injecting 1, 2 and 3 μ l of the standard solution into a Varian 2100 gas chromatograph with the following parameters:

Column temperature:	60°C
Injector temperature:	225°C
FID temperature:	250°C
Chart:	6 min/inch
Carrier gas flow:	40 cc/min nitrogen
Attn.:	8×10^{-11}

Then weigh a 5 g sample of the dosed feed to the nearest 0.01 g in a Falcon tube. Extract the sample with 20 ml of diethyl ether by mixing for 15 minutes in a mechanical shaker, followed by centrifugation at 1300 rpm for 10 minutes. Dilute the high dose level (750 ppm) in a 15 ml graduated centrifuge tube by adding a 1 ml aliquot to 4 ml of diethyl ether. Repeat this procedure using undosed animal feed of the same lot used for the preparation of the dosed feed. This extract will be used as a negative control to assure that there are no interfering peaks contributed by the feed itself. Repeat this procedure using undosed animal feed of the same lot which has been spiked in the laboratory with DCPD at corresponding dose levels.

Quantitate the amount of DCPD in solution by comparing to the calibration curve previously prepared.

Calculate the ppm of DCPD in the dosed feed or spiked (recovery) sample as follows:

To determine mg of sample injected:

$$\frac{5 \text{ g feed}}{20 \text{ ml ether}} = \frac{250 \text{ mg feed}}{1.0 \text{ ml ether}}$$

$$\frac{250 \text{ mg feed}}{1.0 \text{ ml ether}} \times \text{Dilution Factor} = \frac{(x) \text{ mg feed}}{1.0 \text{ ml ether}}$$

3. EXPERIMENTAL DESIGN (Continued)

Dilution Factor = 1 for 80 ppm level
= 0.2 for 750 ppm level

$$\frac{(x) \text{ mg feed}}{1.0 \text{ ml ether}} \times \frac{\mu\text{l sample}}{1000} = \text{mg of feed injected}$$

Calculate the intercept and slope from standard curve as determined by linear regression correlation.

$$\frac{\text{peak response (peak height)} - \text{intercept}}{\text{slope}} = \text{ng of DCPD injected}$$

To determine ppm:

$$\frac{\text{ng of DCPD found}}{\text{mg of feed injected}} = \text{ppm}$$

Determine method of recovery from spiked samples as follows:

$$\text{percent recovery} = \frac{\text{ppm found} \times 100}{\text{ppm added}}$$

Correct the result of the dosed feed sample for method recovery of its corresponding spiked sample.

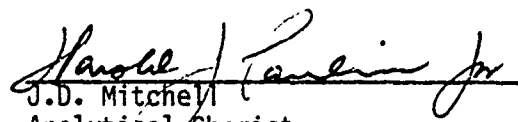
$$\text{corrected ppm} = \frac{\text{sample ppm} \times 100}{\text{percent recovery}}$$

4. RESULTS

DCPD samples were analyzed on a weekly basis by the method previously described. Samples were received by the analytical laboratory and frozen until day of analysis. This action was required due to the volatile nature of the compound. Results of the analysis are indicated in Table 1.

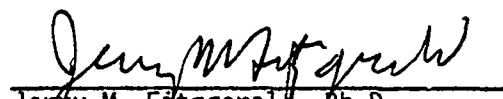
For the 100 ppm level, the average value obtained was 97.7 ± 7.2 ppm, which corresponds to $97.7 \pm 7.2\%$ of the theoretical value. For the 300 ppm level, the average value obtained was 303 ± 15 ppm, which corresponds to $101 \pm 0.5\%$ of the theoretical value. For the 1000 ppm level, the average value obtained was 991 ± 55 ppm, which is equivalent to $99.1 \pm 5.5\%$ of the theoretical value.

Submitted by:


J.D. Mitchell
Analytical Chemist

03/04/80
Date

Reviewed by:


Jerry M. Fitzgerald, Ph.D.
Head, Analytical
Chemistry Section

3/4/80
Date

Approved by:


James Liverman, Ph.D.
Acting Director
Department of Chemistry

3/4/80
Date

TABLE II-F-52

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 1

WEEKLY DCPD FEED ANALYSIS

<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>DOSAGE (PPM)</u>	<u>ANALYSIS VALUE (PPM)^a</u>
05/10/78	05/12/78	R0034K78	0	0
		R0035K78	100	90.1
		R0036K78	300	303
		R0037K78	1000	946
05/18/78	05/19/78	R0049K78	0	0
		R0050K78	100	96.1
		R0051K78	300	304
		R0052K78	1000	996
05/25/78	05/26/78	R0062K78	0	0
		R0063K78	100	94.7
		R0064K78	300	303
		R0065K78	1000	919
06/01/78	06/02/78	R0072K78	0	0
		R0073K78	100	106
		R0074K78	300	280
		R0075K78	1000	931
06/08/78	06/11/78	R0089K78	0	0
		R0090K78	100	107
		R0091K78	300	325
		R0092K78	1000	1011
06/14/78	06/19/78	R0097K78	0	0
		R0098K78	100	98.4
		R0099K78	300	321
		R0100K78	1000	992
06/21/78	06/28/78	R0117K78	0	0
		R0118K78	100	93.4
		R0119K78	300	281
		R0120K78	1000	1037
06/28/78	07/05/78	R0136K78	0	0
		R0137K78	100	103
		R0138K78	300	296
		R0139K78	1000	996
07/05/78	07/08/78	R0150K78	0	0
		R0151K78	100	105.2
		R0152K78	300	285
		R0153K78	1000	1019

^aAll values have been corrected for respective method recovery, run simultaneously with analysis.

TABLE II-F-52 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 1 (CONTINUED)

WEEKLY DCPD FEED ANALYSIS

<u>MIX DATE</u>	<u>ANALYSIS DATE</u>	<u>SAMPLE NUMBER</u>	<u>DOSAGE (PPM)</u>	<u>ANALYSIS VALUE (PPM)^a</u>
07/12/78	07/16/78	R0180K78	0	0
		R0181K78	100	101
		R0182K78	300	314
		R0183K78	1000	915
07/19/78	07/24/78	R0189K78	0	0
		R0190K78	100	92.6
		R0191K78	300	279
		R0192K78	1000	952
07/26/78	07/28/78	R0223K78	0	0
		R0224K78	100	100
		R0225K78	300	309
		R0226K78	1000	1061
08/02/78	08/04/78	R0263K78	0	0
		R0264K78	100	107
		R0265K78	300	320
		R0266K78	1000	1095
08/09/78	08/13/78	R0323K78	0	0
		R0324K78	100	82.2
		R0325K78	300	300
		R0326K78	1000	945

^aAll values have been corrected for respective method recovery, run simultaneously with analysis.

JAMES M. CLINTON, V. M. D.
300 BROOKMEAD DRIVE
CHERRY HILL, NEW JERSEY 08034

AREA CODE 609
TELEPHONE 429-7798

Litton Bionetic Inc.
Project 10734-09
Robert P. Beliles, Ph.D.
Elliot Gordon, Ph.D.

9 January 1978

OPHTHALMOSCOPIC SUMMARY

Both eyes of 34 Beagle dogs were examined by focal illumination, indirect ophthalmoscopy and, when indicated, slit-lamp microscopy. Mydriasis was produced with 1% tropicamide (Mydriacyl 1%, Alcon, expir. date July, 1979). The examination was conducted in a darkened room 229, and darkness maintained until the following day. The dogs examined were eartag numbered as follows:

Male dog

GB77
NW77
LS77
LT77
KK77
LI77
OL77
MK77
PI77
MM77
FF77
DI77
OJ77
QS77
NY77
PK77
MB77

Female dog

LL77
OU77
EZ77
JL77
LO77
HO77
PF77
FJ77
AS77
EI77
AR77
IT77
OH77
IR77
GL77
FO77
LB77

Female dog JL77 has a bilateral epiphora, mainly in the left eye, and female dog HO77 has a slight mucinous exudate in each ventral conjunctival sac. Such findings occasionally precede abnormalities in the precorneal tear film. Both eyes of these two dogs are normal now, however. The findings are not likely to be of major significance in a 90 day study, but because of the known association between the ocular discharge and subsequent changes in the precorneal tear film, I would recommend that if dogs JL77 and HO77 are used, they not be used in the high dose group. The remaining 32 dogs are ophthalmoscopically normal and clinically visual.

James M. Clinton

JAMES M. CLINTON, V. M. D.
300 BROOKMEAD DRIVE
CHERRY HILL, NEW JERSEY 08034

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Litton Bionetics Inc.
Project 10734-09
Robert P. Beliles, Ph. D.
D. Djurickovic, D.V.M.

11 August 1978

FINAL
OPHTHALMOSCOPIC SUMMARY

Both eyes of 16 male and 16 female adult Beagle dogs were examined by focal illumination, indirect ophthalmoscopy and, when indicated, slit-lamp microscopy. Mydriasis was produced with 1% tropicamide (1% Mydracil, Alcon, lot ZJC, Oct. 1979) and the eyes examined in a darkened room. Dr. Djurickovic participated fully in the examinations.

The following dogs were ophthalmoscopically normal:

434, 417, 418, 408, 425, 426, 429, 431, 407, 419, 420, 412, 414, 436, 424, 427,
406, 415, 433, 409, 437, 423, 416, 404, 421, 411 and 435.

Ocular abnormalities were found in the following dogs:

<u>Dog</u>	<u>Sex</u>	<u>Observations</u>
422	F	Left eye: ghost vessels emanating from the limbus, 9 to 2 o'clock
432	F	Right eye: cluster of punctate superficial corneal opacities
405	F	Left eye: cluster of superficial punctate opacities forming a band in the central cornea
430	F	Left eye: Occasional ghost vessels in temporal quadrant of cornea
413	M	Right eye: cluster of superficial punctate paracentral corneal opacities

COMMENTS

Both eyes of all 32 dogs are visual. The ghost vessels in 422 and 430 indicate a prior but now inactive keratitis. The ghost vessels are permanent and should be detectable histologically. The superficial corneal opacities may be sequelae to corneal trauma and should gradually disappear. The lesions observed in the five dogs described above are minor.

At this juncture, I do not believe that the compound being evaluated according to this protocol produces ocular changes in dogs.

James M. Clinton
James M. Clinton, V.M.D.

SUBJECT: FINAL PATHOLOGY REPORT
Mammalian Toxicological Evaluation of DIMP and DCPD
LBI Project No. 10734-09

This report summarizes histopathologic findings in a total of sixteen dogs. Four each males and females, were given 1000 ppm of DCPD in the diet for 3 months. Eight similar dogs served as controls. Tissues collected at necropsy were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5-6 microns and stained with hematoxylin and eosin by standard methods. The following tissues were examined histopathologically: unusual lesions, brain, spinal cord, pituitary gland, sciatic nerve, kidneys, adrenal glands, liver, spleen, pancreas, lung, heart, stomach, small intestine, colon, cecum, mesenteric lymph node, gallbladder, urinary bladder, skin and mammary gland, skeletal muscle, thyroid, testes with epididymis or ovary, prostate or uterus, eye, rib junction and sternum. At least one of the latter two specimens contained bone marrow.

There were few lesions in this group of dogs and none were considered to be related to administration of the compound. Several dogs in both the high dose and control groups had small inflammatory foci in the lungs. Some of these lesions were associated with aspirated foreign material (probably dry food). One dog had an intact, apparently viable nematode in the lung, and there was a minimal inflammatory reaction associated with the parasite. In the majority of the dogs it was not possible to determine the precise cause of the inflammatory lesions in the lungs.

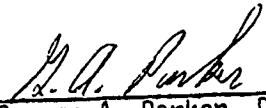
A few dogs in both groups had small cysts in the pituitary glands. These cysts, considered to be cystic Rathke's pouch remnants, are quite common in dogs and are of no clinical significance.

One female in the high dose group had chronic active inflammation of the kidney, renal pelvis and urinary bladder. Such pyelonephritis and cystitis are relatively common spontaneous lesions in dogs and usually are produced by various bacterial pathogens in the lower urinary tract.

Mild hydrocephalus was noted when the brain of one high dose female was trimmed for embedding. This relatively common spontaneous condition often is of unknown etiology, as in the present case. It appears most commonly in young dogs such as those used in this study.


All other lesions were insignificant incidental findings or part of spontaneous disease complexes of dogs. This study indicates that DCPD administered at 1000 ppm in the diet for 3 months produces no discernible histopathologic lesions in dogs.

SUBMITTED BY:


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27 Dec 1978
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TABLE II-F-53

LITTON BIOLOGICS, INC.
PROJECT NO. 10734-09

TABLE I

INCIDENCE OF HISTOLOGIC FINDINGS

GROUP NUMBER SEX NUMBER OF ANIMALS EXAMINED	GROUP 1 MALES 4	GROUP 4 MALES 4	GROUP 1 FEMALES 4	GROUP 4 FEMALES 4
BRAIN Hydrocephalus Mineralization, periaarteriolar, focal	4*	4*	4*	4*
PITUITARY GLAND Cyst(s)	4*	4* 2	4* 3	4* 1
KIDNEYS Fibrosis, interstitial Inflammation, nonsuppurative	4*	4*	4*	4*
LIVER Eosinophilic leukocyte infiltrate	4*	4*	4*	4*
LUNGS Inflammation, interstitial Granuloma, mineralized Parasitism, nematodiasis	4* 2 - -	4* 1 1 -	4* 3 - -	4* 4 - 1
HEART Lymphocytic inflammatory infiltrate	4* 1	4* -	4* -	4* -
SMALL INTESTINE Eosinophilic leukocyte infiltrate	4* -	4* 1	4* -	4* -
MESENTERIC LYMPH NODE Eosinophilic leukocyte infiltrate	4* 1	4* 1	4* -	4* -
URINARY BLADDER Hemorrhage, acute Inflammation, nonsuppurative Hyperplasia, mucosal	4* - - -	4* - - -	4* - - -	4* 1 1 1

* Number of tissues examined.

TABLE II-F-53 (Continued)

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-09

TABLE I (continued)

GROUP NUMBER SEX	GROUP 1 MALES	GROUP 4 MALES	GROUP 1 FEMALES	GROUP 4 FEMALES
NUMBER OF ANIMALS EXAMINED	4	4	4	4
THYROID	3*	4*	4*	4*
Cystic remnant, ultimobranchial duct	-	-	-	-
PARATHYROID (NOT TAKEN ROUTINELY)	-	-	-	-
Cystic remnant, ultimobranchial duct	-	-	-	-

* Number of tissues examined.

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LEGEND TO SUMMARY OF HISTOLOGIC FINDINGS

- = negative finding in a designated tissue or organ; tissue examined.
- + = positive finding (ungraded lesion) encountered in a designated tissue or organ.
- NP = glandular tissue not present in plane of section.
- NR = tissue not received for trimming.
- 1 = positive finding graded "minimal".
- 2 = positive finding graded "mild".
- 3 = positive finding graded "moderate".

TABLE II-F-54

LITTON BIOMETRICS, INC.
PROJECT NO. 10734-09

TABLE 2

SUMMARY OF HISTOLOGIC FINDINGS

GROUP NUMBER SEX ANIMAL NUMBER	GROUP 1 MALES			418	413	GROUP 4 MALES			420	GROUP 1 FEMALES			423	GROUP 4 FEMALES		
	408	411	417			416	419	425		426	434	435		432	436	
BRAIN hydrocephalus Mineralization, periaarteriolar, focal																
SPINAL CORD																
PITUITARY GLAND Cyst(s)																
SCIATIC NERVE																
KIDNEYS Fibrosis, interstitial, radial Inflammation, nonsuppurative																
ADRENAL GLANDS																
LIVER Eosinophilic leukocyte infiltrate, focal																
SPLEEN																
PANCREAS																
LUNGS Inflammation, interstitial, multifocal Inflammation, interstitial, focal Granuloma, mineralized Parasitism, nematodiasis																

TABLE II-F-54 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 2 (continued)

GROUP NUMBER SEX	ANIMAL NUMBER	GROUP 1 MALES		GROUP 4 MALES		GROUP 1 FEMALES		GROUP 4 FEMALES							
		408	411	413	416	419	420	425	426	434	435	423	432	436	436
HEART															
Lymphocytic inflammatory infiltrate, focal, branch of coronary artery			2												
STOMACH															
SMALL INTESTINE															
Eosinophilic leukocyte infiltrate, focal										1					
COLON															
CECUM															
MESENTERIC LYMPH NODE															
Eosinophilic leukocyte infiltrate, focal			1							1					
GALLBLADDER															
URINARY BLADDER															
Hemorrhage, acute, multifocal															2
Inflammation, nonsuppurative, multifocal															2
Mucosal hyperplasia															2
MAMMARY AND SKIN															
			NP												
MUSCLE															
THYROID															
Cystic remnant, ultimobranchial duct			NR												+

TABLE II-F-54 (Continued)

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

TABLE 2 (continued)

GROUP NUMBER	SEX	ANIMAL NUMBER	GROUP 1 MALES	408	411	417	418	413	416	419	420	425	426	434	435	423	432	436	437	GROUP 4 FEMALES

PROSTATE

TESTES

EPIDIDYIMIDES

UTERUS

OVARIES

EYE

RIB JUNCTION

STERNUM

PARATHYROID (NOT TAKEN ROUTINELY)
Cystic remnant, ultimobranchial duct

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 408
DOSAGE: 0 ppm
DATE OF DEATH: 8/15/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6108
GROUP NUMBER: 1
SPECIES: Dog
SEX: Male

GROSS FINDINGS:

Lungs - pleural adhesions, left side.
Thyroids - left thyroid not located; was not in typical location.

MICROSCOPIC FINDINGS:

Lungs - inflammation, interstitial, multifocal, minimal.

MISSING TISSUES:

Thyroids - not received for trimming.
Mammary and skin - glandular tissue not present in plane of section.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 411
DOSAGE: 0 ppm
DATE OF DEATH: 8/15/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6109
GROUP NUMBER: 1
SPECIES: Dog
SEX: Male

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Lungs - inflammation, interstitial, focal, minimal.
Mesenteric lymph node - eosinophilic leukocyte infiltrate, focal,
minimal.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 417
DOSAGE: 0 ppm
DATE OF DEATH: 8/16/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6110
GROUP NUMBER: 1
SPECIES: Dog
SEX: Male

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Liver - eosinophilic leukocyte infiltrate, focal, minimal.
Heart - lymphocytic inflammatory infiltrate, focal, mild, branch of coronary artery.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 418
DOSAGE: 0 ppm
DATE OF DEATH: 8/16/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6111
GROUP NUMBER: 1
SPECIES: Dog
SEX: Male

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
All tissues essentially normal.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>413</u>	PM NUMBER:	<u>78/6116</u>
DOSAGE:	<u>1000 ppm</u>	GROUP NUMBER:	<u>4</u>
DATE OF DEATH:	<u>8/15/78</u>	SPECIES:	<u>Dog</u>
DEATH:	<u>Terminal sacrifice</u>	SEX:	<u>Male</u>
METHOD OF KILL:	<u>Somlethol</u>		

GROSS FINDINGS:

Mesenteric lymph node - minute white nodules along nodes; also reddened.

MICROSCOPIC FINDINGS:

Mesenteric lymph node - essentially normal; white nodules noted in mesenteric lymph nodes at necropsy probably were germinal centers.

MISSING TISSUES:

Mammary and skin - glandular tissue not present in plane of section.

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PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 416
DOSAGE: 1000 ppm
DATE OF DEATH: 8/16/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6117
GROUP NUMBER: 4
SPECIES: Dog
SEX: Male

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Lungs - inflammation, interstitial, focal, mild.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 419
DOSAGE: 1000 ppm
DATE OF DEATH: 8/17/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6118
GROUP NUMBER: 4
SPECIES: Dog
SEX: Male

GROSS FINDINGS:

All tissues appear normal.

MICROSCOPIC FINDINGS:

Pituitary gland - cyst.

Mesenteric lymph node - eosinophilic leukocyte infiltrate, focal,
minimal.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>420</u>	PM NUMBER:	<u>78/6119</u>
DOSAGE:	<u>1000 ppm</u>	GROUP NUMBER:	<u>4</u>
DATE OF DEATH:	<u>8/17/78</u>	SPECIES:	<u>Dog</u>
DEATH:	<u>Terminal sacrifice</u>	SEX:	<u>Male</u>
METHOD OF KILL:	<u>Somlethol</u>		

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Pituitary gland - cysts.
Lungs - granuloma, mineralized, minimal.
Small intestine - eosinophilic leukocyte infiltrate, focal, minimal.

MISSING TISSUES:
Mammary and skin - glandular tissue not present in plane of section.

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PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 425
DOSAGE: 0 ppm
DATE OF DEATH: 8/15/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6112
GROUP NUMBER: 1
SPECIES: Dog
SEX: Female

GROSS FINDINGS:
Mesenteric lymph node - reddened.

MICROSCOPIC FINDINGS:
Pituitary gland - cyst.
Mesenteric lymph node - essentially normal.

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PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 426
DOSAGE: 0 ppm
DATE OF DEATH: 8/16/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6113
GROUP NUMBER: 1
SPECIES: Dog
SEX: Female

GROSS FINDINGS:

Mesenteric lymph node - reddened.

MICROSCOPIC FINDINGS:

Pituitary gland - cyst.

Lungs - inflammation, interstitial, multifocal, mild, associated with foreign material.

Comment - the mesenteric lymph node was histologically normal.

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PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 434
DOSE: 0 ppm
DATE OF DEATH: 8/17/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6114
GROUP NUMBER: 1
SPECIES: Dog
SEX: Female

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Pituitary gland - cyst.
Lungs - inflammation, interstitial, multifocal, minimal.

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PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 435
DOSAGE: 0 ppm
DATE OF DEATH: 8/17/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6115
GROUP NUMBER: 1
SPECIES: Dog
SEX: Female

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Lungs - inflammation, interstitial, multifocal, mild.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER:	<u>423</u>	PM NUMBER:	<u>78/6120</u>
DOSAGE:	<u>1000 ppm</u>	GROUP NUMBER:	<u>4</u>
DATE OF DEATH:	<u>8/15/78</u>	SPECIES:	<u>Dog</u>
DEATH:	<u>Terminal sacrifice</u>	SEX:	<u>Female</u>
METHOD OF KILL:	<u>Somlethol</u>		

GROSS FINDINGS:

Kidneys - both appear scarry.
Urinary bladder - numerous small red spots about 1mm in measurement.

MICROSCOPIC FINDINGS:

Brain - hydrocephalus, mild; mineralization, periarteriolar, focal, minimal.
Kidneys - fibrosis, interstitial, radial, moderate, bilateral, cortex and medulla; inflammation, nonsuppurative, moderate, bilateral, papilla and pelvis.
Lungs - inflammation, interstitial, multifocal; mild, associated with foreign material.
Urinary bladder - hemorrhage, acute, multifocal, mild; inflammation, nonsuppurative, multifocal, mild; with mild mucosal hyperplasia.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 432
DOSAGE: 1000 ppm
DATE OF DEATH: 8/15/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6121
GROUP NUMBER: 4
SPECIES: Dog
SEX: Female

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Lungs - inflammation, interstitial, multifocal, mild.
Thyroids - cystic ultimobranchial duct remnant.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 436
DOSAGE: 1000 ppm
DATE OF DEATH: 8/16/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6122
GROUP NUMBER: 4
SPECIES: Dog
SEX: Female

GROSS FINDINGS:

Pituitary gland - small cyst.
Uterus - mild mucometra.

MICROSCOPIC FINDINGS:

Pituitary gland - cyst.
Lungs - parasitism, nematodiasis, mild; inflammation, interstitial, focal, minimal.
Uterus - no lesion recognized; the slight increase in uterine fluid noted at necropsy was due to normal cyclic secretory activity.

LITTON BIONETICS, INC.
PROJECT NO. 10734-09

INDIVIDUAL ANIMAL PATHOLOGY EVALUATION

ANIMAL NUMBER: 437
DOSAGE: 1000 ppm
DATE OF DEATH: 8/16/78
DEATH: Terminal sacrifice
METHOD OF KILL: Somlethol

PM NUMBER: 78/6123
GROUP NUMBER: 4
SPECIES: Dog
SEX: Female

GROSS FINDINGS:
All tissues appear normal.

MICROSCOPIC FINDINGS:
Lungs - inflammation, interstitial, multifocal, mild.
Parathyroids - cystic ultimobranchial duct remnant.

A P P E N D I X

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